



Health, EFSA. P. O. A., (AHAW), W., More, S., Bøtner, A., Butterworth, A., Calistri, P., Depner, K., Edwards, S., Garin-Bastuji, B., Good, M., Gortázar Schmidt, C., Michel, V., Miranda, M. A., Nielsen, S. S., Raj, M., Sihvonen, L., Spooler, H., Stegeman, J. A., Thulke, H-H., ... Bicout, D. (2017). Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): *Batrachochytrium salamandrivorans* (Bsal). *EFSA Journal*, 15(11), e05071-n/a. <https://doi.org/10.2903/j.efsa.2017.5071>

Publisher's PDF, also known as Version of record

License (if available):  
CC BY

Link to published version (if available):  
[10.2903/j.efsa.2017.5071](https://doi.org/10.2903/j.efsa.2017.5071)

[Link to publication record in Explore Bristol Research](#)  
PDF-document

This is the final published version of the article (version of record). It first appeared online via EFSA at <http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2017.5071/abstract?systemMessage=Wiley+Online+Library+usage+report+download+page+will+be+unavailable+on+Friday+24th+November+2017+at+21%3A00+EST+%2F+02.00+GMT+%2F+10%3A00+SGT+%28Saturday+25th+Nov+for+SGT+>. Please refer to any applicable terms of use of the publisher.

## University of Bristol - Explore Bristol Research

### General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available: <http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

ADOPTED: 25 October 2017

doi: 10.2903/j.efsa.2017.5071

**Assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429):**  
***Batrachochytrium salamandrivorans (Bsal)***

EFSA Panel on Animal Health and Welfare (AHAW),  
Simon More, Anette Bøtner, Andrew Butterworth, Paolo Calistri, Klaus Depner,  
Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortázar Schmidt,  
Virginie Michel, Miguel Angel Miranda, Søren Saxmose Nielsen, Mohan Raj, Liisa Sihvonen,  
Hans Spoolder, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde,  
Preben Willeberg, Christoph Winckler, Francesca Baldinelli, Alessandro Broglia,  
Denise Candiani, Chiara Fabris, Gabriele Zancanaro, Beatriz Beltrán-Beck, Lisa Kohnle and  
Dominique Bicot

**Abstract**

*Batrachochytrium salamandrivorans (Bsal)* has been assessed according to the criteria of the Animal Health Law (AHL), in particular criteria of Article 7 on disease profile and impacts, Article 5 on the eligibility of *Bsal* to be listed, Article 9 for the categorisation of *Bsal* according to disease prevention and control rules as in Annex IV, and Article 8 on the list of animal species related to *Bsal*. The assessment has been performed following a methodology composed of information collection and compilation, expert judgement on each criterion at individual and, if no consensus was reached before, also at collective level. The output is composed of the categorical answer, and for the questions where no consensus was reached, the different supporting views are reported. Details on the methodology used for this assessment are explained in a separate opinion. According to the assessment performed, *Bsal* can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL. The disease would comply with the criteria as in sections 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (d) and (e) of Article 9(1). The assessment here performed on compliance with the criteria as in Section 1 of Annex IV referred to in point (a) of Article 9(1) is inconclusive. The animal species to be listed for *Bsal* according to Article 8 (3) criteria are species of the families Salamandridae and Plethodontidae as susceptible and Salamandridae and Hynobiidae as reservoirs.

© 2017 European Food Safety Authority. *EFSA Journal* published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

**Keywords:** *Batrachochytrium salamandrivorans*, *Bsal*, Animal Health Law, listing, categorisation, impact

**Requestor:** European Commission

**Question number:** EFSA-Q-2017-00428

**Correspondence:** alpha@efsa.europa.eu

**Panel members:** Dominique Bicout, Anette Bøtner, Andrew Butterworth, Paolo Calistri, Klaus Depner, Sandra Edwards, Bruno Garin-Bastuji, Margaret Good, Christian Gortázar Schmidt, Virginie Michel, Miguel Angel Miranda, Simon More, Søren Saxmose Nielsen, Mohan Raj, Liisa Sihvonen, Hans Spooler, Jan Arend Stegeman, Hans-Hermann Thulke, Antonio Velarde, Preben Willeberg and Christoph Winckler.

**Acknowledgements:** The Panel wishes to thank An Martel for the support provided to this scientific output.

**Suggested citation:** EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More S, Bøtner A, Butterworth A, Calistri P, Depner K, Edwards S, Garin-Bastuji B, Good M, Gortázar Schmidt C, Michel V, Miranda MA, Nielsen SS, Raj M, Sihvonen L, Spooler H, Stegeman JA, Thulke H-H, Velarde A, Willeberg P, Winckler C, Baldinelli F, Broglia A, Candiani D, Fabris C, Zancanaro G, Beltrán-Beck B, Kohnle L and Bicout D, 2017. Scientific Opinion on the assessment of listing and categorisation of animal diseases within the framework of the Animal Health Law (Regulation (EU) No 2016/429): *Batrachochytrium salamandrivorans* (Bsal). EFSA Journal 2017;15(11):5071, 34 pp. <https://doi.org/10.2903/j.efsa.2017.5071>

**ISSN:** 1831-4732

© 2017 European Food Safety Authority. EFSA Journal published by John Wiley and Sons Ltd on behalf of European Food Safety Authority.

This is an open access article under the terms of the [Creative Commons Attribution-NoDerivs](#) License, which permits use and distribution in any medium, provided the original work is properly cited and no modifications or adaptations are made.

Reproduction of the images listed below is prohibited and permission must be sought directly from the copyright holder:

Figure 1 and Figure 1 (Annex): © Wouter Beukema, Ghent University; Figure 2 (left) and Figure 2 (left; Annex): © An Martel, Ghent University; Figure 2 (right) and Figure 2 (right; Annex): © Frank Pasmans, Ghent University; Figures 3, 4 and 3 (Annex): © Frank Pasmans, Ghent University



The EFSA Journal is a publication of the European Food Safety Authority, an agency of the European Union.



## Table of contents

Abstract.....	1
1. Introduction.....	4
1.1. Background and Terms of Reference as provided by the requestor.....	4
1.2. Interpretation of the Terms of Reference.....	4
2. Data and methodologies.....	4
3. Assessment.....	4
3.1. Assessment according to Article 7 criteria.....	4
3.1.1. Article 7(a) Disease Profile.....	4
3.1.1.1. Article 7(a)(i) Animal species concerned by the disease.....	5
3.1.1.2. Article 7(a)(ii) The morbidity and mortality rates of the disease in animal populations.....	6
3.1.1.3. Article 7(a)(iii) The zoonotic character of the disease.....	7
3.1.1.4. Article 7(a)(iv) The resistance to treatments, including antimicrobial resistance.....	7
3.1.1.5. Article 7(a)(v) The persistence of the disease in an animal population or the environment.....	7
3.1.1.6. Article 7(a)(vi) The routes and speed of transmission of the disease between animals, and, when relevant, between animals and humans.....	8
3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union, where the disease is not present in the Union, the risk of its introduction into the Union.....	9
3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools.....	10
3.1.2. Article 7(b) The impact of diseases.....	12
3.1.2.1. Article 7(b)(i) The impact of the disease on agricultural and aquaculture production and other parts of the economy.....	12
3.1.2.2. Article 7(b)(ii) The impact of the disease on human health.....	12
3.1.2.3. Article 7(b)(iii) The impact of the disease on animal welfare.....	12
3.1.2.4. Article 7(b)(iv) The impact of the disease on biodiversity and the environment.....	13
3.1.3. Article 7(c) Its potential to generate a crisis situation and its potential use in bioterrorism.....	14
3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures.....	14
3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities.....	15
3.1.4.2. Article 7(d)(ii) Vaccination.....	15
3.1.4.3. Article 7(d)(iii) Medical treatments.....	16
3.1.4.4. Article 7(d)(iv) Biosecurity measures.....	16
3.1.4.5. Article 7(d)(v) Restrictions on the movement of animals and products.....	20
3.1.4.6. Article 7(d)(vi) Killing of animals.....	21
3.1.4.7. Article 7(d)(vii) Disposal of carcasses and other relevant animal by-products.....	21
3.1.5. Article 7(e) The impact of disease prevention and control measures.....	22
3.1.5.1. Article 7(e)(i) The direct and indirect costs for the affected sectors and the economy as a whole.....	22
3.1.5.2. Article 7(e)(ii) The societal acceptance of disease prevention and control measures.....	23
3.1.5.3. Article 7(e)(iii) The welfare of affected subpopulations of kept and wild animals.....	23
3.1.5.4. Article 7(e)(iv) The environment and biodiversity.....	24
3.2. Assessment according to Article 5 criteria.....	24
3.2.1. Outcome of the assessment of <i>Batrachochytrium salamandrivorans</i> according to criteria of Article 5(3) of the AHL on its eligibility to be listed.....	25
3.3. Assessment according to Article 9 criteria.....	25
3.3.1. Non-consensus-questions.....	28
3.3.2. Outcome of the assessment of criteria in Annex IV for <i>Batrachochytrium salamandrivorans</i> for the purpose of categorisation as in Article 9 of the AHL.....	29
3.4. Assessment of Article 8.....	30
4. Conclusions.....	31
References.....	32
Abbreviations.....	34

## 1. Introduction

### 1.1. Background and Terms of Reference as provided by the requestor

The background and Terms of Reference (ToR) as provided by the European Commission for the present document are reported in Section 1.2 of the scientific opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9, and 8 within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

### 1.2. Interpretation of the Terms of Reference

The interpretation of the ToR is as in Section 1.2 of the scientific opinion on the ad hoc methodology followed for the assessment of the disease to be listed and categorised according to the criteria of Article 5, Annex IV according to Article 9, and 8 within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

The present document reports the results of assessment on *Batrachochytrium salamandrivorans* (Bsal) according to the criteria of the AHL articles as follows:

- Article 7: Bsal profile and impacts
- Article 5: eligibility of Bsal to be listed
- Article 9: categorisation of Bsal according to disease prevention and control rules as in Annex IV
- Article 8: list of animal species related to Bsal.

## 2. Data and methodologies

The methodology applied in this opinion is described in detail in a dedicated document about the ad hoc method developed for assessing any animal disease for the listing and categorisation of diseases within the Animal Health Law (AHL) framework (EFSA AHAW Panel, 2017).

## 3. Assessment

### 3.1. Assessment according to Article 7 criteria

This section presents the assessment of Bsal according to the Article 7 criteria of the AHL and related parameters (see Table 2 of the opinion on methodology (EFSA AHAW Panel, 2017)), based on the information contained in the fact-sheet as drafted by the selected disease scientist (see Section 2.1 of the scientific opinion on the ad hoc methodology) and amended by the AHAW Panel.

#### 3.1.1. Article 7(a) Disease Profile

A recently described fungus, *Batrachochytrium salamandrivorans* (Bsal), is causing several die-offs in salamander populations in Europe (Martel et al., 2013; Spitzen-van der Sluijs et al., 2016). This chytridiomycete fungus, which belongs to the order Rhizophydiales, produces two types of spores, motile (zoo-)spores and non-motile encysted spores (Stegen et al., 2017), and causes the lethal skin disease chytridiomycosis in salamanders and newts (Caudata, tailed amphibians). Chytridiomycosis due to Bsal is characterised by multifocal superficial erosions and extensive epidermal ulcerations all over the body. Coinciding clinical signs include excessive shedding of the skin, anorexia, apathy, ataxia and death (Martel et al., 2013). This fungus is pathogenic for most western Palearctic salamander and newt taxa and is considered a major threat to the region's biodiversity (Martel et al., 2013; Spitzen-van der Sluijs et al., 2016). Salamanders can be resistant (no infection, no disease), tolerant (infection in the absence of disease), moderately susceptible (infection resulting in clinical disease with the possibility of subsequent recovery) or highly susceptible (infection resulting in lethal disease). It is not known which factors underpin the susceptibility/resistance of a species. Infection experiments demonstrated that frogs and toads are not susceptible to the disease, but can act as healthy carriers (Stegen et al., 2017).

Bsal is believed to originate from Asia where it appears to be endemically present (Martel et al., 2014; Laking et al., 2017) and from where it was presumably imported into Europe through the trade of live amphibians.

### 3.1.1.1. Article 7(a)(i) Animal species concerned by the disease

#### *Susceptible animal species*

##### Parameter 1 – Naturally susceptible wildlife species (or family/orders)

Fire salamander (*Salamandra salamandra*) is the index species, with several confirmed cases of mass mortality in the wild in Belgium, Germany and the Netherlands (Spitzen-van der Sluijs et al., 2016). *Bsal* infection was implicated in the death of Alpine newts (*Ichthyosaura alpestris*) and smooth newts (*Lissotriton vulgaris*) in Belgium, Germany and the Netherlands (Spitzen-van der Sluijs et al., 2016). The following caudata species have died in captivity due to confirmed and natural *Bsal* infection: *I. alpestris*, *Salamandra algira*, *S. salamandra* (including the subspecies *alfredschmidti*, *almanzoris*, *bernardezi*, *fastuosa*, *gallaica*, *gigliolii*, *salamandra*, *terrestris*, *wernerii*), *Salamandra corsica*, *Salamandra infraimmaculata*, *Triturus macedonicus*, *Triturus marmoratus*, *Notophthalmus viridescens*, *Taricha granulosa* (Sabino-Pinto et al., 2015; Martel and Pasmans, 2017).

##### Parameter 2 – Naturally susceptible domestic species (or family/orders)

No farmed species are known affected, only captive species (see Parameter 1) are considered susceptible.

##### Parameter 3 – Experimentally susceptible wildlife species (or family/orders)

Experimental infection with a single dose of  $10^5$  zoospores of *Bsal* resulted in 100% mortality in specimens from the following western Palearctic species, when kept at a constant temperature of 15°C: *S. salamandra*, *Lissotriton italicus*, *L. vulgaris*, *I. alpestris*, *Triturus cristatus*, *Speleomantes strinatii*, *N. viridescens*, *Taricha granulosa*, *Pleurodeles waltl*, *Salamandrina terdigitata*, *Neurergus crocatus*, *Euproctus platycephalus*. Based on phylogeny, the vast majority of western Palearctic caudata species is considered to be susceptible to *Bsal* infection (Martel et al., 2014). Very few European species (e.g. *Lissotriton helveticus*) appear to be resistant to infection. A recent study by Stegen et al. (2017) demonstrated pronounced differential susceptibility between species. Highly susceptible species such as the fire salamander die even with a very low infectious dose. Disease course in the moderately susceptible Alpine newt depends on infectious dose: a high dose results in mortality, whereas exposure to a low dose results in highly variable scenarios, from persistent infections with eventual clearance of the fungus, to lethal infections. Based on *Bsal* infection dynamics in experimental infection trials (Martel et al., 2014), Stegen et al. (2017) predict at least the following western Palearctic caudata taxa to be highly susceptible: all species belonging to the genera *Salamandra*, *Euproctus*, *Neurergus* and *Pleurodeles*. Given close relatedness to the highly susceptible genus *Salamandra*, species of the genera *Chioglossa*, *Lyciasalamandra* and *Mertensiella* are predicted to be highly susceptible as well. Based on *Bsal* infection dynamics, field data and phylogeny, species belonging to the following western Palearctic genera are predicted to be moderately susceptible (i.e. disease outcome is dependent on infectious dose): *Lissotriton*, *Ichthyosaura*, *Triturus*, *Salamandrina*, *Speleomantes*, *Ommatotriton* (Martel et al., 2014; Stegen et al., 2017). Susceptibility of the genera *Proteus* and *Calotriton* cannot be estimated based on lack of experimental and field data and phylogenetic extrapolations.

Based on infection dynamics in experimentally infected animals and field data, Asian caudata belonging to the genera *Tylototriton*, *Paramesotriton*, *Hypselotriton* and *Cynops* are considered moderately susceptible (Martel et al., 2014; Laking et al., 2017) and at least these species are considered natural reservoirs of *Bsal* infection in Asia. At least some Asian salamander species belonging to the family Hynobiidae (genera *Salamandrella*, *Hynobius*, *Onychodactylus*) are considered tolerant (i.e. can be persistently infected in the absence of clinical signs and pathology) (Martel et al., 2014).

Susceptibility of American caudata species is largely unknown. Based on results from experimental infection trials, *Bsal* is capable of causing mortality in species belonging to the genera *Notophthalmus* and *Taricha* (Martel et al., 2014). Susceptibility of the largest caudata family (Plethodontidae) is currently unknown, although *Bsal* was shown capable of invading the skin of at least one species (*Plethodon glutinosus*) (Martel et al., 2014). Species belonging to the family Sirenidae might be considered tolerant to infection with *Bsal* (Martel et al., 2014).

##### Parameter 4 – Experimentally susceptible domestic species (or family/orders)

No farmed species known affected, only captive species (see Parameter 1) are considered susceptible.



## Reservoir animal species

### Parameter 5 – Wild reservoir species (or family/orders)

Asian caudata are generally regarded as natural hosts of *Bsal* and potential reservoirs. *Bsal* infections in apparently asymptomatic wild Asian caudata have been confirmed in *Cynops pyrrhogaster*, *Cynops ensicauda*, *Tylototriton vietnamensis*, *Tylototriton asperrimus*, *Tylototriton ziegleri*, *Tylototriton uyenoi*, *Salamandrella keyserlingii*, *Paramesotriton deloustali*, *Hynobius nebulosus* and *Onychodactylus japonicus* (Martel et al., 2014; Laking et al., 2017). *Bsal* was found present in a museum specimen of *C. ensicauda* dating from 1861 (Martel et al., 2014).

Moderately susceptible European caudata, such as *I. alpestris*, may equally serve as reservoir hosts (Stegen et al., 2017): when exposed to a low infectious dose, this species can be persistently infected, shedding significant spore numbers, without showing any evidence of acquired immunity that protects against re-infection.

Although originally not considered susceptible to infection, anuran (frogs and toads) have recently been shown to be potential hosts, which can transfer infections to salamanders, acting as healthy carriers (Stegen et al., 2017).

*Bsal* was shown to be present on wild small-webbed fire-bellied toads (*Bombina microdeladigitata*) from Vietnam and on representatives of the same species that have recently been imported in Germany (Laking et al., 2017).

### Parameter 6 – Domestic reservoir species (or family/orders)

No farmed species known affected, only captive species (see Parameter 1) are considered susceptible.

## 3.1.1.2. Article 7(a)(ii) The morbidity and mortality rates of the disease in animal populations

### Morbidity

#### Parameter 1 – Prevalence/Incidence

Thus far, *Bsal* has been demonstrated in 15 wild populations of salamanders in Belgium, Germany and the Netherlands (Spitzen-van der Sluijs et al., 2016) and in five populations in captivity in Germany (1), the Netherlands (2), Spain (1) and the United Kingdom (1) (Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016). The total number of wild and captive populations of salamanders in Europe is not known.

The prevalence of *Bsal* in an infected salamander population varies according to the scenario. During a *Bsal* outbreak in fire salamanders (*S. salamandra*), the *Bsal* prevalence varied between 25% and 63% (Stegen et al., 2017). Across a 10-day interval, the infection probability in fire salamanders was estimated at 0.33 (Stegen et al., 2017).

In Vietnam, part of the presumed region of origin of *Bsal*, supposed *Bsal* endemism coincides with a much lower prevalence of 3% (Laking et al., 2017). Although proper prevalence studies are lacking, the low number of *Bsal* positive wild salamanders in China, Japan and Thailand (17 out of 432 samples, 3.9%) corroborates low but consistent prevalence of *Bsal* in natural populations in Asia (Martel et al., 2014).

#### Parameter 2 – Case-morbidity rate (% clinically diseased animals out of infected ones)

In natural infections in captivity and in the wild, case-morbidity depends on the species involved and, at least for some species, on the infectious dose. For naturally infected animals of the genus *Salamandra* (both in captives and in wild animals), case-morbidity approaches 100%. Asymptomatic infections with *Bsal* in species belonging to this genus have not been confirmed so far. In experimental infection trials (Martel et al., 2014) conducted in some western Palearctic species, exposure to a single high dose ( $10^4$  spores/animal) of *Bsal* resulted in a case-morbidity of 100%, except for the palmate newt (*L. helveticus*), which might be resistant to infection. However, exposure to a low dose (100 spores/animal) resulted in a lower case-morbidity of 6/9 in the moderately susceptible Alpine newt (*I. alpestris*), while remaining 100% in the highly susceptible fire salamander (Stegen et al., 2017), suggesting case-morbidity to be a function of infectious dose in moderately susceptible species but not in highly susceptible species (for a presumed list of highly and moderately susceptible species, see Section 3.1.1.1).

In the supposed natural reservoir hosts (Asian caudata species), the case-morbidity rate also appears to depend on the infectious dose. While exposure to a single, high infectious dose resulted in a case-morbidity rate of 100% in experimental infection trials in the species *P. deloustali*, *C. pyrrhogaster*, *Tylototriton wenxianensis* and *Hypselotriton cyanurus* (Martel et al., 2014), natural infections in Asian caudata belonging to the genera *Paramesotriton* and *Tylototriton* either in the wild or in captivity could not be linked to any clinical signs or decreased body condition (Martel et al., 2014; Laking et al., 2017). Probably, infection pressure in natural populations is low (as corroborated by the low prevalence, see above), exposing animals to low doses of *Bsal* under natural conditions.

Few species were designated as tolerant: the eastern Asiatic *Salamandrella keyserlingii* and the North American *Siren intermedia* (Martel et al., 2014), with a supposed case-morbidity rate of 0%.

### Mortality

#### Parameter 3 – Case-fatality rate

As for the case-morbidity rate, the case-fatality rate of *Bsal* infections appears to be highly dependent on host species and infectious dose. Fire salamanders show a 100% case-fatality rate, deduced from field data (Stegen et al., 2017), infection trials (Martel et al., 2014) and data from outbreaks in captive animals (Martel and Pasmans, 2017). The case-fatality rate for this species is independent of the infectious dose (Stegen et al., 2017). During an outbreak in the wild, survival of infected fire salamanders was sixfold lower than survival in non-infected salamanders (Stegen et al., 2017), resulting in loss of over 99.9% of the population (Spitzen-van der Sluijs et al., 2016). Other species with predicted similarly high case-fatality rates that are dose independent include the western Palearctic genera that are listed as highly susceptible in Section 3.1.1.1. Mortality rates in Alpine newts have been shown to be highly dependent on infectious dose, ranging from 1/5 animals exposed to a single, low dose of *Bsal* to 5/5 animals exposed to a single, high dose (Stegen et al., 2017). Western Palearctic species that have been listed as moderately susceptible in Section 3.1.1.1 probably follow a similar pattern of dose dependent mortality. How this translates to reduced survival in the wild and population declines is currently not clear for these moderately susceptible species.

As for the case-morbidity rate, the case-fatality rate of *Bsal* infections in Asian salamanders of the above-mentioned (Section 3.1.1.2) species, probably strongly depends on infectious dose. When exposed to a single, high *Bsal* dose, 4/8 *C. pyrrhogaster*, 3/4 *P. deloustali* and 3/5 *H. cyanurus* died. Mortality due to *Bsal* has not been reported in Asian caudate, neither in the wild nor in captivity, suggesting the case-fatality rate in natural infections to be low in these species.

Experimental infection trials and field data suggested a very low case-fatality rate in some caudata considered tolerant after successful infection with a single, high dose of *Bsal*: *Salamandrella keyserlingii* and *Siren intermedia* (Martel et al., 2014).

#### 3.1.1.3. Article 7(a)(iii) The zoonotic character of the disease

##### Presence

#### Parameter 1 – Report of zoonotic human cases (anywhere)

Human infection has never been reported. To date, there are no report of zoonotic cases linked to *Bsal* infection, which is considered not zoonotic.

#### 3.1.1.4. Article 7(a)(iv) The resistance to treatments, including antimicrobial resistance

#### Parameter 1 – Resistant strain to any treatment even at laboratory level

None reported.

#### 3.1.1.5. Article 7(a)(v) The persistence of the disease in an animal population or the environment

##### Animal population

#### Parameter 1 – Duration of infectious period in animals

The infectious period, here defined as the period during which detectable amounts of *Bsal* can be demonstrated in skin swabs from infected salamanders depends on the host species, the infectious dose and the environmental conditions (Stegen et al., 2017). Differences in the infectious period between *Bsal* strains cannot be excluded at this point. When exposed to a single high dose, highly susceptible species such as fire salamanders (see Section 3.1.1.1) generally die within 15 days after



exposure at a constant temperature of 15°C, whereas at 4°C or when exposed to a lower *Bsal* dose, the duration of *Bsal* shedding and time to mortality are significantly longer (Stegen et al., 2017). Moderately susceptible species (see Section 3.1.1.1) can develop chronic infections and shed *Bsal* for at least 4 months (Martel et al., 2014; Stegen et al., 2017). The duration of the infectious period in natural populations is currently not known.

#### Parameter 2 – Presence and duration of latent infection period

The presence of latent infections (defined as infected but not yet infectious) has not been demonstrated yet.

#### Parameter 3 – Presence and duration of the pathogen in healthy carriers

The existence of healthy pathogen carriers (they are infectious but asymptomatic) has been hypothesised to mainly occur in the region of *Bsal* origin, Asia (Laking et al., 2017). The duration of the infection in healthy carriers in nature is not known. Experimental infection of species that may be healthy carriers in the wild (*Cynops*, *Paramesotriton*) demonstrates shedding of *Bsal* by infected animals for up to 4 months after exposure to a single high dose of *Bsal*, at a constant temperature of 15°C. Based on infection trials, healthy carriers may be common in moderately susceptible European caudata species as well (Stegen et al., 2017), in which *Bsal* was demonstrated up to more than 3 months post-exposure. Since this long-term persistence did not result in protection against re-infection, these species may prove suitable *Bsal* carriers for long periods of times (Stegen et al., 2017). Infections experiments also demonstrated European anura (e.g. *Alytes*) can act as healthy carriers (Stegen et al., 2017).

#### *Environment*

#### Parameter 4 – Length of survival (dpi) of the agent and/or detection of DNA in selected matrices (soil, water, air) from the environment (scenarios: high and low T)

*Bsal* produces encysted spores that float on water and are the infective and resistant form of the agent in the environment, (Stegen et al., 2017). Survival has been assessed in pond water where spores remain infectious for at least 31 days at 15°C.

Infected salamanders contaminate forest soil, which can transmit infection to naïve animals. *Bsal* DNA was detected in forest soil up to 200 days in experimentally inoculated soil samples. Contaminated forest soil remains infective for at least 48 h (Stegen et al., 2017).

### **3.1.1.6. Article 7(a)(vi) The routes and speed of transmission of the disease between animals, and, when relevant, between animals and humans**

#### *Routes of transmission*

#### Parameter 1 – Types of routes of transmission from animal to animal (horizontal, vertical)

Vertical transmission is likely in species that produce metamorphosed offspring (*Salamandra atra* and *Salamandra lanzai*, some *S. salamandra* subspecies) but this needs further investigation. Larvae of fire salamanders could not be experimentally infected with *Bsal* (Van Rooij et al., 2015). The tipping point when larval salamanders become susceptible during metamorphosis is not known. Horizontal transmission within and between caudata species has been demonstrated (Martel et al., 2014) but also from anurans to fire salamanders (Stegen et al., 2017). Transmission is likely to occur during animal–animal contact (e.g. during courtship, territorial interactions) and indirectly by encysted spores floating on water, motile zoospores or by contaminated forest soil (Stegen et al., 2017). Adherence of encysted spores to inert matrices (e.g. scales on bird feet) may promote large distance spread (Stegen et al., 2017).

#### Parameter 2 – Types of routes of transmission between animals and humans (direct, indirect, including food-borne)

Not applicable because no infections in humans have been reported.

#### *Speed of transmission*

#### Parameter 3 – Incidence between animals and, when relevant, between animals and humans

Salamanders carrying high infection loads can spread *Bsal* infection to naïve salamanders within 2 h of cohousing (Martel et al., 2014).

Parameter 4 – Transmission rate (beta) (from  $R_0$  and infectious period) between animals and, when relevant, between animals and humans

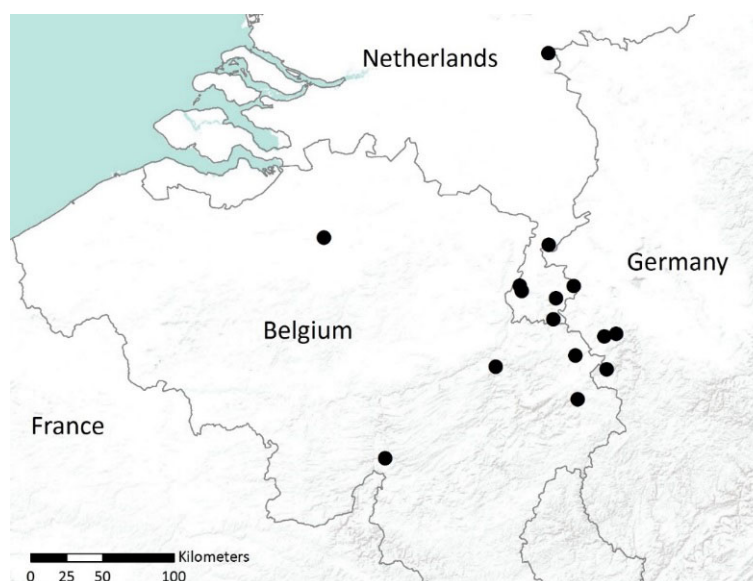
Infection probability in a naturally infected fire salamander population across a 10-day interval was estimated at 0.33 (CI: 0.169–0.512) (Canessa et al., 2017).

**3.1.1.7. Article 7(a)(vii) The absence or presence and distribution of the disease in the Union, where the disease is not present in the Union, the risk of its introduction into the Union**

*Presence and distribution*

Parameter 1 – Map where the disease is present in EU

The disease has been detected in collections of captive salamanders (Germany, the Netherlands, Spain and the United Kingdom (Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016)) and in natural populations (Belgium, Germany, the Netherlands) of salamanders. A map of the currently known localities of affected natural populations is presented in Figure 1.



**Figure 1:** Reported outbreaks of *Bsal* infections in natural populations of salamanders from 2013 to 2017 (author: Wouter Beukema, Ghent University)

Parameter 2 – Type of epidemiological occurrence (sporadic, epidemic, endemic) at MS level

All occurrences presently known in natural populations are linked to mass mortality events with population declines in at least Belgium, Germany and the Netherlands. *Bsal* among wild amphibians in Europe is more widely distributed than previously known, which can either indicate recent spread of the fungus or identified infected sites that were previously undetected (Spitzen-van der Sluijs et al., 2016).

*Risk of introduction*

Parameter 3 – Routes of possible introduction

Precise routes of introduction are currently not known and thus this paragraph is largely hypothetical. A likely route of introduction is through the trade in live, infected amphibians (Martel et al., 2014; Laking et al., 2017). Other sources (e.g. aquatic plants) cannot be excluded, given the presence of an environmentally resistant life stage of *Bsal*. Direct or indirect contact with native caudata may result in *Bsal* introduction: direct contact, e.g. by release of infected, captive caudata in garden ponds containing native caudata; indirect contact, e.g. by the use of fomites (buckets, containers, dipnets, etc.) both for care-taking of pet caudata and in field activities involving native caudata or by disposal of contaminated terrarium content (soil, water) in caudata habitat.

Introduction in naïve regions from outbreak areas may be possible through (a) expansion via overlapping amphibian populations (b) movement by humans of infected animals and (c) mechanical

vectors (wildlife, humans (contaminated clothes), fomites (materials and machines used during field activities), contaminated water and/or soil).

#### Parameter 4 – Number of animal moving and/or shipment size

No official quantitative data are available with regard to movements of amphibians for the trade (research, exhibitions, zoo, aquaria, etc.) within the European Union (EU) or between the EU and third countries given the lack of a unique harmonised system commodity code identifier. Of the most widely available pet newt species (*Hypselotriton orientalis*), 2.3 million newts have been imported into the USA between 2001 and 2009 (Herrel and van der Meijden, 2014). This species is a potential carrier of *Bsal*. Similar numbers have likely been imported in the EU. Other Asian caudata that may be potential carriers of *Bsal* and that regularly turn up in the EU trade are species of the genera *Pachytriton*, *Paramesotriton*, *Cynops*, *Tylotriton* and *Salamandrella*. Approximately 156,000 salamanders, mostly including shipments with *Bsal* risk, are reported to be annually imported into the USA (Yap et al., 2015). In 2013, 21,000 individuals (*Paramesotriton chinensis*, *N. viridescens* and *Cynops* spp.) were imported into the Netherlands (Spitzen-van der Sluijs et al., 2015).

#### Parameter 5 – Duration of infectious period in animal and/or commodity

Depending on the species, infectious dose, temperature, the duration of the infectious period is ranging from 2 weeks to more than 4 months (see Section 3.1.1.5). Infected soil remains infective for at least 2 days, pond water for at least 1 month (Stegen et al., 2017).

#### Parameter 6 – List of control measures at border (testing, quarantine, etc.)

None currently installed.

#### Parameter 7 – Presence and duration of latent infection and/or carrier status

The existence of asymptomatic pathogen carriers has been hypothesised to mainly occur in the region of *Bsal* origin, Asia (Laking et al., 2017). The duration of the infection in asymptomatic carriers in nature is not known. Experimental infection of species that may be asymptomatic carriers in the wild (*Cynops*, *Paramesotriton*) demonstrate shedding of *Bsal* by infected animals for up to 4 months after exposure to a single high dose of *Bsal*, at a constant temperature of 15°C.

#### Parameter 8 – Risk of introduction

The trade in live animals likely constitutes a constant threat of introduction of *Bsal* and other amphibian pathogens. However, *Bsal* prevalence in trade is likely low. Of 2,335 samples of captive amphibians examined, only 3 were positive for *Bsal* (Martel et al., 2014). It is therefore expected that only the importation of large numbers of potential *Bsal* carrier species into the EU constitutes a significant risk.

The risk of other potential, non-amphibian routes of entry can currently not be estimated.

### **3.1.1.8. Article 7(a)(viii) The existence of diagnostic and disease control tools**

#### *Diagnostic tools*

##### Parameter 1 – Existence of diagnostic tools

Non-invasive sampling can be performed on live amphibians by collecting skin swabs for the highly specific detection of *Bsal* DNA (using quantitative polymerase chain reaction (qPCR)) (Blooï et al., 2013). The limit of detection of the fungus is 0.1 genomic equivalents (GE). The samples that can be analysed by qPCR include swabs, toe clips and skin samples (Blooï et al., 2013). The qPCR is able to detect the fungus before the animal shows clinical signs of disease (Martel et al., 2014).

Alternatives to qPCR include histopathology of skin, immunohistochemistry using polyclonal antibodies or the use of a recently developed lateral-flow technique (Dillon et al., 2017). These methods fail to discriminate between *Bsal* and its sister species *Batrachochytrium dendrobatidis* (Bd) and have a lower sensitivity than the qPCR method (Dillon et al., 2017).

#### *Control tools*

##### Parameter 2 – Existence of control tools

*Bsal* in captive caudata can be controlled using a combination of proper quarantine and entry control for *Bsal* of any newly acquired animal. Quarantine should consist of a period of at least 40 days, preferably at a *Bsal* permissive temperature (optimum of 15°C) (Martel et al., 2013) during

which overall health of the animal should be assessed and a skin swab should be collected for quantification of *Bsal* DNA using qPCR during the last week of the quarantine period (Blooï et al., 2015b). During this period, all materials that have come into contact (directly or indirectly) with the quarantined animal should be properly disinfected. This can be done by chemical decontamination. An overview of effective disinfection protocols is given in Table 1. Hydrogen peroxide shows poor activity against *Bsal*. Heat treatment is to be expected to result in fast killing of all life stages of *Bsal* but needs further study. The fungus poorly tolerates high temperatures: *Bsal* cultures are killed after incubation for 5 days at 25°C (Blooï et al., 2015a). If *Bsal* responds to heat as its sister species *Bd*, exposing materials to 60°C for 5 min or 100°C for 1 min should be efficient (Johnson et al., 2003).

**Table 1:** Minimal exposure time for 100% killing of *Bsal* spores and sporangia at room temperature (Van Rooij et al., 2017)

Disinfectant	Concentration	Minimal exposure time for 100% killing of <i>Bsal</i>
<b>Ethanol (EtOH)</b>	70%	30 s
<b>Disolol®</b>	Undiluted	30 s
<b>Hibiscrub®</b>	0.25, 0.5, 0.75%	30 s
<b>Chloramine-T®</b>	0.5%	5 min
	1%	2 min
<b>Bleach</b>	1:5 dilution	5 min
	4%	30 s
<b>Kickstart®</b>	0.05%	5 min
	0.1%	2 min
<b>Potassium permanganate (KMnO<sub>4</sub>)</b>	1%	10 min
	2%	5 min
<b>Virkon S®</b>	0.5%	5 min
	1%	2 min
<b>Dettol medical®</b>	1:20 dilution	5 min
<b>Biocidal®</b>	Undiluted	30 s
<b>Safe4®</b>	Undiluted	30 s
<b>F10®</b>	1:100 dilution	30 s
	1:250 dilution	30 s
	1:500 dilution	30 s
	1:1,000 dilution	30 s
<b>Sodium chloride (NaCl)</b>	10%	10 min

*Bsal*: *Batrachochytrium salamandrivorans*.

If salamanders are infected by *Bsal* in captivity, the infection can be effectively treated either using temperature treatment (Blooï et al., 2015b) or chemotherapeutics (Blooï et al., 2015a), which are capable of clearing the infection. Collections of captive caudata can be cleared from *Bsal* infection (Martel and Pasmans, 2017) by keeping the infected salamanders at 25°C for 10 days (Blooï et al., 2015a). For salamander species that do not tolerate these relatively high temperatures, an alternative consists on the combination of topical application of voriconazole with polymyxin E and keeping the infected salamanders at 20°C for 10 days (Blooï et al., 2015a). Post-treatment assessment of *Bsal* absence is obligatory and the treatment may need repeating until total clearance.

Controlling *Bsal* in natural populations of salamanders is currently limited to measures that prevent *Bsal* introduction. No curative measures are available to mitigate *Bsal* in natural caudata populations once the pathogen is established. As an emergency measure to safeguard highly valuable populations from extinction, the development of captive assurance colonies is recommended (Stegen et al., 2017).

### 3.1.2. Article 7(b) The impact of diseases

#### 3.1.2.1. Article 7(b)(i) The impact of the disease on agricultural and aquaculture production and other parts of the economy

*The level of presence of the disease in the Union*

Parameter 1 – Number of MSs where the disease is present

##### **Currently**

In wild populations: Belgium, Germany, the Netherlands (Spitzen-van der Sluijs et al., 2016).

In captive salamanders: Germany, the Netherlands, Spain, the United Kingdom, (Fitzpatrick et al., 2016; Sabino-Pinto et al., 2015).

##### **Potential**

In wild populations: recent modelling of *Bsal* infection and disease across Europe based on its native niche (Beukema et al., 2017) predicts the potential of *Bsal* to affect caudata populations in all European nations, with high suitability of regions that are of special conservation interest for caudata (regions with high caudata endemism such as the Iberian peninsula, Corsica and Sardinia, Italy and the Alps and the countries bordering the Adriatic sea).

In captive salamanders: *Bsal* has the potential to spread between collections among all European countries (and beyond), probably mainly through traffic in live caudata.

*The loss of production due to the disease*

Parameter 2 – Proportion of production losses (%) by epidemic/endemic situation

Not applicable because salamanders are not used as production animals.

#### 3.1.2.2. Article 7(b)(ii) The impact of the disease on human health

No zoonotic cases have been reported.

*Bsal* most probably does not infect humans, given the low thermal preference of the fungus.

#### 3.1.2.3. Article 7(b)(iii) The impact of the disease on animal welfare

Parameter 1 – Severity of clinical signs at case level and related level and duration of impairment

In highly susceptible hosts and at permissive temperatures, *Bsal* infections invariably result in a lethal, ulcerative skin disease (Figure 2), with morbidity and mortality approaching 100% (Stegen et al., 2017) and over 99.9% reduction of the population size (Spitzen-van der Sluijs et al., 2016).

In moderately susceptible hosts, the infection results in skin infection, either or not coinciding with cutaneous disease (skin ulceration) and potentially leading to death (Martel et al., 2014; Stegen et al., 2017).

Tolerant hosts do not show obvious signs of reduced welfare after infection.





#### 3.1.2.4. Article 7(b)(iv) The impact of the disease on biodiversity and the environment

Parameter 1 – Endangered wild species affected: listed species as in CITES and/or IUCN list

**Table 2:** Tentative list of presumed moderately or highly susceptible European caudata species either included in Annex IV of the Habitats Directive and/or listed in the European Red List of Amphibians

<b>Species name</b>	<b>Inclusion in Annex IV, Habitats directive</b>	<b>IUCN European Red List</b>	<b>Estimated susceptibility</b>
<i>Lyciasalamandra helverseni</i>	Yes	VU	High
<i>Atylodes genei</i>	Yes	VU	Moderate
<i>Calotriton arnoldi</i>	Yes	CR	Unknown
<i>Calotriton asper</i>	Yes	NT	Unknown
<i>Chioglossa lusitanica</i>	Yes	VU	High
<i>Euproctus montanus</i>	Yes	LC	High
<i>Euproctus platycephalus</i>	Yes	EN	High
<i>Lissotriton italicus</i>	Yes	LC	High
<i>Lissotriton montandoni</i>	Yes	LC	Unknown
<i>Pleurodeles waltl</i>	No	NT	High
<i>Proteus anguinus</i>	Yes	VU	High
<i>Salamandra algira</i> *	No	VU	High
<i>Salamandra atra</i>	Yes	LC	High
<i>Salamandra lanzai</i>	Yes	VU	High
<i>Salamandrina perspicillata</i>	Yes	LC	Moderate
<i>Salamandrina terdigitata</i>	Yes	LC	Moderate
<i>Speleomantes ambrosii</i>	Yes	NT	Moderate
<i>Speleomantes flavus</i>	Yes	VU	Moderate

Species name	Inclusion in Annex IV, Habitats directive	IUCN European Red List	Estimated susceptibility
<i>Speleomantes imperialis</i>	Yes	NT	Moderate
<i>Speleomantes italicus</i>	Yes	NT	Moderate
<i>Speleomantes sarraabusensis</i>	Yes	VU	Moderate
<i>Speleomantes supramontis</i>	Yes	EN	Moderate
<i>Speleomantes strinatii</i>	Yes	NT	Moderate
<i>Triturus carnifex</i>	Yes	LC	Moderate
<i>Triturus cristatus</i>	Yes	LC	Moderate
<i>Triturus dobrogicus</i>	Yes	NT	Moderate
<i>Triturus ivanbureschi</i>	Yes		Moderate
<i>Triturus macedonicus</i>	Yes		Moderate
<i>Triturus marmoratus</i>	Yes	LC	Moderate
<i>Triturus pygmaeus</i>	Yes	NT	Moderate

CR: critically endangered; EN: endangered; VU: vulnerable; NT: near threatened; LC: least concern.

\*: It does not naturally occur in the EU.

#### Parameter 2 – Mortality in wild species

In natural populations, mortality due to *Bsal* has been confirmed extensively in fire salamanders (*S. salamandra*), with crashes reducing the populations by over 99.9% (Spitzen-van der Sluijs et al., 2016). Fire salamander populations undergo a 90% decline within 6 months after the onset of the disease (Stegen et al., 2017). Mortality has been observed in newts (*I. alpestris*, *L. vulgaris*) but the impact of *Bsal* on natural newt populations is currently unclear.

#### Environment

#### Parameter 3 – Capacity of the pathogen to persist in the environment and cause mortality in wildlife

*Bsal* is a wildlife pathogen causing extinction events in fire salamander populations (Martel et al., 2013; Stegen et al., 2017) and probable extinction in several highly susceptible and threatened European species (see Section 3.1.1.1 and Table 1). For persistence in the environment, see Section 3.1.1.5 Parameter 4.

### 3.1.3. Article 7(c) Its potential to generate a crisis situation and its potential use in bioterrorism

#### Parameter 1 – Listed in OIE/CFSPH classification of pathogens

This is a recently discovered pathogenic fungus (Martel et al., 2013), recently added to the OIE list of aquatic animal diseases.<sup>1</sup> Further, it is not included in the Center for Food Security and Public Health (CFSPH) list of Bioterrorism and High Consequence Pathogen (CFSPH, 2017).

#### Parameter 2 – Listed in the Encyclopaedia of Bioterrorism Defence of Australia Group

It is not listed.

#### Parameter 3 – Included in any other list of potential bio- agro-terrorism agents

It is not listed.

### 3.1.4. Article 7(d) The feasibility, availability and effectiveness of the following disease prevention and control measures

In general, control measures are applicable to captive animals, to a lesser or no extent to wild animals.

<sup>1</sup> [http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre\\_batrachochytrium\\_dendrobatidis.htm](http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_batrachochytrium_dendrobatidis.htm)

### 3.1.4.1. Article 7(d)(i) Diagnostic tools and capacities

#### Availability

##### Parameter 1 – Officially/internationally recognised diagnostic tool, OIE certified

No OIE certified diagnostic tools are available. The only available qPCR protocol currently routinely used internationally as the reference standard for Bsal detection in skin and skin swabs is the one developed by Blooi et al. (2013) that has been used in several laboratories providing consistent results (Sabino-Pinto et al., 2015; Fitzpatrick et al., 2016; Spitzen-van der Sluijs et al., 2016). The diagnostic characteristics of this test has been recently evaluated by Thomas et al. (submitted) and, despite some limitations linked to the limited sample size used for its validation and being based on a single laboratory study, study results are reported below under 'effectiveness'.

#### Effectiveness

##### Parameter 2 – Se and Sp of diagnostic test

The diagnostic sensitivity (DSe), diagnostic specificity (DSp) and reproducibility of the Bsal qPCR developed by Blooi et al. (2013) were evaluated using DNA samples from 26 experimentally infected and 12 non-infected salamanders in three external labs (see above under 'availability'). Exact binomial confidence intervals were obtained using the test results in the three laboratories using the method of Clopper–Pearson (Brown et al., 2001). The DSe was 96% (95% IC: 80.4–99.9%) in one laboratory and 100% (95% IC: 86.6–100%) in two laboratories. The DSp in all three laboratories was 12 out of 12 (100%; 95% IC: 73–100) (estimates were based on data used in Thomas et al. (submitted), i.e. the dichotomous outcome of the test performed in three different laboratories).

#### Feasibility

##### Parameter 3 – Type of sample matrix to be tested (blood, tissue, etc.)

In live animals, non-invasively collected samples (skin swabs) can be easily and quickly collected (Blooi et al., 2013; Martel et al., 2013, 2014). A cotton-tipped swab should be rubbed firmly over the abdominal area, ventral tail and foot 10 times each and subsequently stored dry, and preferably frozen to avoid DNA degradation.

In dead animals, qPCR on skin tissue can be combined with histopathology of the skin and, if available, immunohistochemistry (Van Rooij et al., 2015; White et al., 2016).

### 3.1.4.2. Article 7(d)(ii) Vaccination

#### Availability

##### Parameter 1 – Types of vaccines available on the market (live, inactivated, DIVA, etc.)

None.

##### Parameter 2 – Availability/production capacity (per year)

Not applicable since no vaccines have been developed.

#### Effectiveness

##### Parameter 3 – Field protection as reduced morbidity (as reduced susceptibility to infection and/or to disease)

Vaccination of caudata of Bsal does not seem to be a promising measure for future development. Repeated cycles of experimental infection/treatment in two salamander species (fire salamanders and Alpine newts) did not induce any obvious protection against re-infection and clinical disease (Stegen et al., 2017).

##### Parameter 4 – Duration of protection

Not applicable since no vaccine is available.

#### Feasibility

##### Parameter 5 – Way of administration

Not applicable since no vaccine is available.

### 3.1.4.3. Article 7(d)(iii) Medical treatments

#### Availability

##### Parameter 1 – Types of drugs available on the market

Only infected animals in captivity can be efficiently treated. The treatment of choice consists of heat treatment (25°C for 10 days) (see Section 3.1.1.8 Parameter 2). Cocktails of drugs (topical treatment combining polymyxin E submersion baths and voriconazole sprayed twice a day for 10 days at an ambient temperature of 20°C) can be used (Blooi et al., 2015b).

##### Parameter 2 – Availability/production capacity (per year)

Not applicable since temperature treatment is the preferred option. Voriconazole is preferably used as the intravenous formulation for humans and is not registered for use in amphibians. Polymyxin E is widely used in veterinary medicine.

#### Effectiveness

##### Parameter 3 – Therapeutic effects on the field (effectiveness)

Heat and antimicrobial treatment can be 100% effective in eliminating *Bsal* infection in captive caudata. Since the number of *Bsal* strains available is currently very limited; however, it is not known to which extent these treatment protocols are applicable to *Bsal* in general (for example, a strain with a higher thermal tolerance may survive the heat treatment protocol; sensitivity to antimicrobial drugs may vary between strains).

#### Feasibility

##### Parameter 4 – Way of administration

For caudata species that tolerate the relatively high temperature of 25°C, this is by far the preferred method. Animals can be treated in large groups both in terrestrial and aquatic species, there is no impact on the environment (contamination with antimicrobial drugs) and this procedure may be suitable during quarantine of imported caudata species that tolerate this temperature. Among European species, the ones that could tolerate such high temperature (> 25°C) are at least *Triturus dobrogicus*, usually kept at 15°C, with critical thermal maximum (CT max) of 36.8°C ± 0.2 (Gvozdić et al. 2007); *Ommatotriton vittatus* (kept at 10°C) with CT max of 34.2°C (Warburg 1971) and *S. infraimmaculata* (kept at 10°C) with CT max of 32.5°C (Warburg 1971).

Antimicrobial treatment is laborious, consisting of a labour-intensive protocol of bathing (polymyxin E), spraying (voriconazole) and housing at a temperature of 20°C. Treatment fluids that are disposed of may end up in the environment.

### 3.1.4.4. Article 7(d)(iv) Biosecurity measures

#### Availability

##### Parameter 1 – Available biosecurity measures

Biosecurity measures should be aimed at

##### 1) preventing further introduction of *Bsal* (and other amphibian pathogens) into the EU

No official measures currently exist. Prevention of *Bsal* introduction may be achieved by a combination of quarantine joined with obligatory entry control of any amphibian entering the EU (EFSA, 2017). An importation ban is an alternative approach but may drive amphibian movements across borders. The trade is well aware of *Bsal* and has issued guidelines for their members. In a joint report of the Ornamental Fish International, Ornamental Aquatic Trade Association, the Reptile and Exotic Pet Trade Association and the Pet Industry Federation, they support quarantine measures and testing of animals for entry control, restricting salamander movements for potential *Bsal* hosts, a tracking system for captive caudata, the implementation of biosecurity measures and raising public awareness (Joint Response, 2017). Effective prevention, however, requires knowledge of the route of entry of *Bsal* in Europe. Although traffic in live amphibians is generally considered a major route, further studies about entry routes of chytrid fungus infections are needed.

##### 2) preventing introduction of *Bsal* in naïve regions from outbreak regions

To prevent spill-over from *Bsal* from outbreak areas into naïve areas, field protocols have been put in place, which emphasise proper disinfection of all materials between amphibian populations visited.



The implementation of these protocols is obligatory for field workers who ask for permits in Flanders (see below) and similar biocontrol measures are advised but not officially imposed in the Netherlands (advised by RAVON Foundation – Reptile, Amphibian and Fish Conservation Netherlands) and Germany (advised by Trier University). In Flanders, a perimeter of 1 km has been defined around a *Bsal* locality (Duffel). No permits are being delivered by the authorities for field activities within this perimeter.

Summary of measures imposed by the Flemish government (ANB) for field activities in response to the emergence of *Bsal*:

- Manipulation of amphibians is allowed only when strictly necessary
- Either disposable vinyl gloves should be worn when handling amphibians or hands should be disinfected after handling
- All materials used must be cleaned and disinfected between sites and preferably on-site, including clothes that made contact with the environment. Virkon 1% should be used as disinfectant. Alternatively, heating at 60°C for 30 min is accepted. This measure also applies to:
  - amphibian monitoring activities
  - all activities in amphibian habitats (not necessarily limited to activities pertaining to amphibians)
- Vehicles should be parked on paved roads wherever possible
- Dead or ill amphibians should only be handled with gloves
- For actions targeted at reducing road mortality during spring migration, one dedicated set of material per site should be used
- For education oriented projects, only a single freshwater locality per day can be visited
- For monitoring projects, all materials must be cleaned and disinfected between sites as mentioned before
- For all activities in amphibian habitats (not necessarily limited to activities pertaining to amphibians) all materials must be cleaned and disinfected between sites as mentioned before.

Public education by means of information panels informs visitors to outbreak sites in some areas (for example Robertville in Wallonia, Bunde in the Netherlands: Figure 3). Raising public awareness through general media is important to alert people when dead amphibians are encountered in the field and to encourage using proper hygienic measures before and after visit to amphibian populations. In the countries where *Bsal* outbreaks are currently ongoing in natural populations, all amphibians are strictly protected by law.



**Figure 3:** Information panel at the entrance of the outbreak site in Robertville (Belgium) in French, German and Dutch (author: Frank Pasmans, Ghent University)



Preferably, outbreak sites should be closed for recreational use and for any non-essential activity to limit opportunities of *Bsal* spread through human activities.

The fast identification of a *Bsal* outbreak in the field and the instalment of an early warning system should prevent *Bsal* spread and introduction in naïve regions from outbreak regions. A network of diagnostic laboratories is currently being built across Europe in the framework of EC Tender ENV.B.3/SER/2016/0028. An early warning system (passive and sometimes active monitoring) is in place in Belgium, Germany and the Netherlands and this will be implemented across the EU in the framework of the above mentioned EC Tender ENV.B.3/SER/2016/0028. Passive monitoring consists of the detection and collection of suspect cases/mortality by any stakeholder visiting amphibian habitats (field workers, recreational users, scientists, hunters, etc.), followed by specific examination for the presence of *Bsal*. Passive monitoring appears as the most appropriate method for *Bsal* detection in caudata species as the infection is associated with mortality. Active monitoring consists of detecting *Bsal* in swab samples in amphibian populations.

According to different minimum expected prevalences and population sizes and assuming 100% test Sp, the sample size needed to provide the 95% probability of detecting at least one positive animal is reported in Table 3. Since the test Se was derived using experimentally infected animals, it may be possible that this value may be lower when used in the field with naturally infected populations. For this reason and with the aim of being conservative, 80.4% Se, the lower confidence bound computed using the data from one of the three laboratories (the one with the lowest sensitivity) reported in Section 3.1.4.1, has been used to estimate the sample sizes as in Table 3. Additionally, it needs to be noted that it is not clear whether there exist other fungi or organisms in natural populations that could cross-react with *Bsal*, in which case they would give false positive test results, resulting in specificity lower than 100%. In the estimates provided in Table 3, the effect of dealing with different wild animal species not homogeneously distributed in space has not been taken into account. Therefore, active monitoring, if not designed according to the different factors affecting the population size and the geographical distribution of the host and the pathogen, could have very low probability in detecting *Bsal* in the salamander populations.

**Table 3:** Sample size needed for providing the 95% probability of detecting at least one positive animal according to different population sizes and minimum expected prevalence, assuming 80% test Se and 100% test Sp (NA = not applicable)

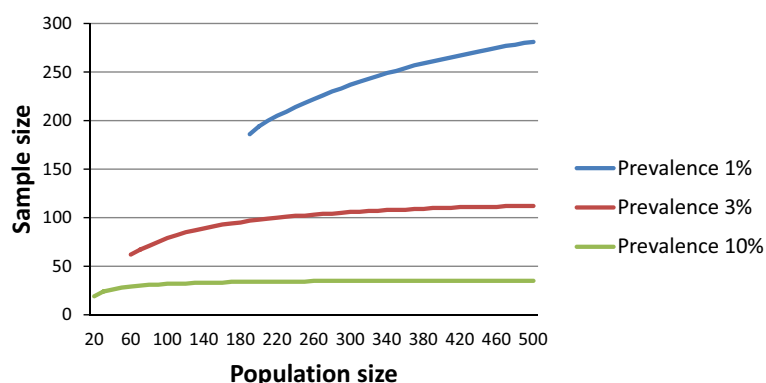
Population size	Minimum expected prevalence		
	1%	3%	10%
<b>19</b>	NA*	NA*	<b>19</b>
<b>62</b>	NA*	<b>62</b>	29
<b>186</b>	<b>186</b>	96	34
<b>200</b>	194	98	34
<b>250</b>	218	102	34
<b>300</b>	237	106	35
<b>350</b>	251	108	35
<b>400</b>	263	110	35
<b>450</b>	273	111	35
<b>500</b>	281	112	35
<b>550</b>	288	113	35
<b>600</b>	294	114	36
<b>100,000 (infinite population size)</b>	373	124	36

In bold, the sample size threshold for having 95% probability of detecting at least one infected animals at 1%, 3% and 10% prevalences.

\*: NA: not applicable as the sample size does not allow demonstrating 95% probability absence of the diseases.

The design prevalence of 1%, 3% and 10% are chosen as examples. Where the disease has an epidemic behaviour as currently in Europe, the prevalence in outbreak area can be expected to be > 10%, whereas 3% could be considered as the minimum expected prevalence value registered in endemic areas as in Asia (see Section 3.1.1.2), thus such a design prevalence could be used for limiting the introduction of the disease into EU from endemic countries, e.g. in consignment of salamanders from Asia.

Similarly, in the graph in Figure 4, it is possible to visualise the trend of sample size according to increasing population size and to the different minimum expected prevalence.



**Figure 4:** Sample size by population size for providing the 95% probability of detecting at least one positive animal according to different minimum expected prevalence (1%, 3% and 10%), assuming 80% test Se and 100% test Sp

Several organisations (e.g. in Belgium Natuurpunt, Natagora, Ghent University and the Flemish and Wallonian government; in the Netherlands RAVON, the Dutch government) collaborate in the establishment of regional hotlines, where suspect cases can be reported.

*Ex situ* conservation has been proposed as the sole effective measure in preventing *Bsal* infected populations of highly susceptible species from going extinct (Stegen et al., 2017). A captive assurance colony of the remaining fire salamanders exists in the Netherlands (collaboration between zoos, RAVON and the Dutch government) and is envisaged in Flanders in case *Bsal* would infect local fire salamander populations.

### 3) preventing *Bsal* spread between captive and natural caudata populations

No official measures currently exist. Any direct or indirect contact between captive and natural caudata populations must be avoided. If European populations of captive caudata would be screened for the presence of *Bsal*, with subsequent follow-up to clear existing infections, this, combined with proper entry control and quarantine, may assure *Bsal* to be absent from captive salamanders, excluding a potentially important source of *Bsal* for native salamanders.

Raising public awareness by means of presentations on meetings and publications in journals that reach a terrarium audience has been intensively pursued: terrarium keepers, associated with the hobby clubs seem well aware of the problem. The largest association of terrarium enthusiasts in Europe (the Deutsche Gesellschaft für Herpetologie und Terrarienkunde (DGHT)) has published a position paper on *Bsal* (DGHT, 2016) in which they advise their members to strictly adhere to biosecurity measures, monitor their captive caudata, urge for fast diagnostics and treatment, quarantine and entry control to limit spread of *Bsal* through captive caudata. The Dutch 'Salamandervereniging', comprising caudata keepers only, advertises similar measures to their members. Overall, the terrarium keepers are highly motivated to eliminate any *Bsal* infection from captivity given the near total destruction of susceptible species in captive caudata collections once *Bsal* entered (Martel and Pasmans, 2017).

Building veterinary capacity across Europe to increase the likelihood that *Bsal* infections in captivity are diagnosed and treated correctly and quickly should be considered a priority. A network of diagnostic laboratories is currently being built across Europe in the framework of the EC Tender ENV.B.3/SER/2016/0028.

### Effectiveness

#### Parameter 2 – Effectiveness of biosecurity measures in preventing the pathogen introduction

Biosecurity measures are expected to be crucial in preventing pathogen introduction in natural and captive amphibian populations in naïve regions.

## Feasibility

### Parameter 3 – Feasibility of biosecurity measures

Feasibility depends on the measures and on the willingness of authorities to implement these measures. Overall, the biosecurity measures proposed above should be easy to implement but conflicts may arise with regional interests (e.g. closure of recreational areas). A further complicating factor is the involvement of non-professionals (the general public, terrarium keepers, and volunteers). For proper implementation of the biosecurity measures, at least the following stakeholders should be motivated to comply with biosecurity measures:

- any person professionally or voluntarily involved in field activities in amphibian habitats (e.g. research, education, habitat restoration, etc.);
- trade through the entire commercial chain;
- captive collection holders (zoos, private keepers, *ex situ* conservation programmes);
- any person visiting outbreak sites (several outbreak sites are in important touristic sites that are heavily frequented for recreational use),

### **3.1.4.5. Article 7(d)(v) Restrictions on the movement of animals and products**

## Availability

### Parameter 1 – Available movement restriction measures

None currently in place.

## Effectiveness

### Parameter 2 – Effectiveness of restriction of animal movement in preventing the between farm spread

Restriction of animal movement is applicable to captive animals and it is highly likely to limit spread of *Bsal* if properly implemented. Release of captive animals in natural amphibian populations should be avoided, unless the released animals have been produced in the framework of a strictly monitored and designed conservation programme taking all necessary biosecurity measures into account, including testing for known amphibian diseases before release. In this respect, the sale of amphibians in garden centres for release in garden ponds should be strongly discouraged or even forbidden.

For amphibians in trade, animal movement of only *Bsal*-free animals would help in preventing the introduction and further spread of this pathogen.

In case *Bsal* would be detected in captive salamanders, all movements of animals to and from the infected collection should be strictly avoided until successful treatment and clearance of the fungus from the collection. A generalised transport restriction of salamanders between captive sites is extremely difficult to monitor, since many species can be easily transported in small containers via any transportation means.

For natural populations, any movement of animals between localities (e.g. for translocation, reintroduction, reinforcement) should preferably be restricted to cases with a clear conservation benefit based on sound scientific evidence. In case of movement, all animals to be moved should be clinically healthy and should be free of at least the amphibian pathogens *Bsal*, Bd and ranaviruses.

## Feasibility

### Parameter 3 – Feasibility of restriction of animal movement

Restriction of animal movement should be feasible especially in professional organisations (trade, zoos, conservation-oriented organisations). Feasibility in non-professionals (e.g. terrarium keepers) depends on implementation (e.g. efforts spent on continuously raising public awareness) and probably also on the level of the keeper: knowledgeable private keepers associated with hobby clubs or internet fora/social media groups are more likely to be informed than non-knowledgeable people buying newts in pet shops for an ornamental aquarium. This latter category is important since the vast amount of Asian newts imported (*H. orientalis*, a potential *Bsal* carrier) most probably ends up with this latter group of keepers who are more difficult to reach.

### 3.1.4.6. Article 7(d)(vi) Killing of animals

#### Availability

##### Parameter 1 – Available methods for killing animals

None. Caudata can be killed by various methods. Injection (intravenously, intracoelomically, intralymphatically) of sodium pentobarbital is deemed acceptable (AVMA, 2013). Topical application using bathing solutions of anaesthetics such as tricaine methanesulfonate or benzocaine are equally considered acceptable and allow euthanising groups of animals (AVMA, 2013). Although previously deemed unacceptable, recent evidence suggests cooling and subsequent freezing of amphibians to result in painless death (Shine et al., 2015) and this method can be applied for mass euthanasia.

#### Effectiveness

##### Parameter 2 – Effectiveness of killing animals (at farm level or within the farm) for reducing/stopping spread of the disease

Killing animals could be carried out at three levels:

- *Bsal*-infected animals in trade: killing in combination with biosecurity measures would effectively stop spread from these animals. However, given the relatively ease and efficacy of the existing treatments and presence of diagnostic tests, treatment should be the preferred option over killing.
- *Bsal*-infected animals in captive collections: idem as for animals in trade.
- *Bsal*-infected animals in natural populations: in theory, removing all infected (or even all) animals from a natural population in combination with biosecurity measures would limit the spread of the disease. However, the presence of non-caudata and environmental *Bsal* reservoirs (Stegen et al., 2017), combined with a low probability of capturing all caudata in a population, renders success of culling all infected animals in natural populations highly unlikely (Stegen et al., 2017). Indeed, given its host population density independent epidemiology, removing only a proportion of a population is highly unlikely to stop an outbreak (Stegen et al., 2017).

#### Feasibility

##### Parameter 3 – Feasibility of killing animals

Feasibility of killing animals in trade or in captive collections can be considered high but may be strongly opposed by public opinion.

Feasibility of killing a sufficient number of animals in natural populations can be considered not feasible for the reasons mentioned above.

### 3.1.4.7. Article 7(d)(vii) Disposal of carcasses and other relevant animal by-products

#### Availability

##### Parameter 1 – Available disposal option

Most dead captive caudata carcasses probably end up in the dustbin together with household waste (although in theory this is generally prohibited). If this waste is subsequently treated (e.g. incineration), this will effectively kill *Bsal*. When disposed of in open air waste dumps, this might hold a theoretical risk of pathogen spread.

A proper disposal option for caudata carcasses and associated wastes is commercial fixed plant incineration. Disposal of carcasses should include a procedure that inactivates *Bsal* (and preferably other amphibian diseases such as Bd and ranaviruses), which can be achieved either by thermal treatment (at least 30 min at 60°C) or chemical disinfection (see above).

Carcasses and associated wastes (e.g. soil or water from terraria) should not be disposed of in nature without prior decontamination, since this may result in spill-over of *Bsal* to native amphibians.

#### Effectiveness

##### Parameter 2 – Effectiveness of disposal option

Proper disinfection procedures should result in safe and effective disposal.

## Feasibility

### Parameter 3 – Feasibility of disposal option

High, since caudata typically are small animals of less than 20 cm, relatively small volumes have to be disposed off. Feasibility of disposal of large quantities of contaminated terrarium content can be an issue.

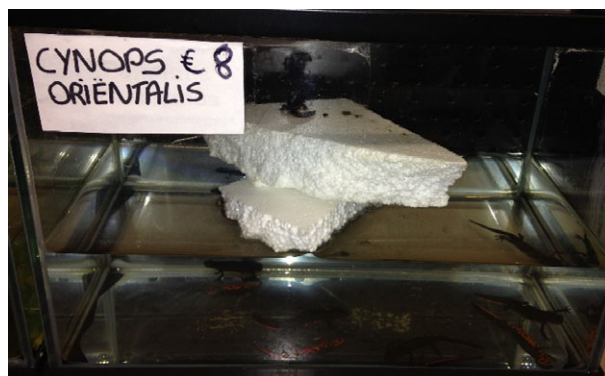
### 3.1.5. Article 7(e) The impact of disease prevention and control measures

#### 3.1.5.1. Article 7(e)(i) The direct and indirect costs for the affected sectors and the economy as a whole

##### Parameter 1 – Cost of control (e.g. treatment/vaccine, biosecurity)

Very difficult to quantify and highly depending on the scenario:

- For trade: combination of entry control, quarantine and, if necessary, treatment. The volume of traded caudata represents a minor proportion of the trade (Gilbert et al., 2016). Costs for *Bsal*-free caudata in the trade can be included in the price setting of the animals. Overall, caudata are relatively inexpensive (customer prices generally between EUR 10 and 100 per animal). The Asian species traded in the largest numbers and a possible vector for *Bsal* (*H. orientalis*) typically sells for low prices (around EUR 10 per animal, see Figure 5).



**Figure 5:** *Hypselotriton* ('*Cynops*') *orientalis* for sale in a garden centre. This species is the most commonly imported Asian salamander and a potential carrier of *Bsal* (author: Frank Pasmans, Ghent University)

- For keepers of caudata: combination of entry control, quarantine, biosecurity measures (e.g. disinfection of terrarium contents) and, if necessary, treatment. A diagnosis based on the combination of necropsy, histopathology and qPCR costs approximately EUR 100 at the diagnostic centre of Ghent University, allowing group diagnosis of the disease in an infected collection. Depending on the diagnostic laboratory, follow-up of infected collections using qPCR on skin swabs costs between EUR 20 and 50 per swab. The preferred method of treatment (heat) is a low-cost treatment. Currently, keepers of caudata (zoos, conservation programmes and private keepers) have shown motivation to cover these costs, given the far-reaching implications of *Bsal* infection for the collection's health.
- For natural populations: preventive biosecurity measures as mentioned above do come with a cost (disinfection procedures, public education, early warning system, closure of recreational areas). Active and passive monitoring is currently being supported by governments (Dutch, German and Belgian governments). Costs for passive monitoring are limited to the transportation of carcasses and diagnostic procedures. Costs for active monitoring cover field activities (sampling) and diagnostic procedures. Costs for captive assurance colonies depend on the strategy followed: from mere maintenance of individuals *ex situ* (requiring staff, infrastructure and material costs) to manage breeding programmes (requiring the same + genetic management).



#### Parameter 2 – Cost of eradication (culling, compensation)

- For captive animals: depending on price setting for compensation/animal. Group treatment or euthanasia for amphibians is relatively low cost. Obligatory follow-up with skin swabs after treatment represents the major cost.
- For natural populations: eradication is currently not considered an option for both technical and ethical concerns (Stegen et al., 2017).

#### Parameter 3 – Cost of surveillance and monitoring

Surveillance and monitoring, both in animals in captivity and the wild, can be done using qPCR on skin swabs. Apart from the costs associated with collecting the samples, costs of qPCR as mentioned above.

#### Parameter 4 – Trade loss (bans, embargoes, sanctions) by animal product

Currently, the USA has banned the importation of caudata based on the risk of introduction of *Bsal* (US Fish and Wildlife Service, 2016). If the EU would install an importation ban or restriction, this is likely not to result in significant economic losses (although, as mentioned before, this cannot be accurately estimated, given the lack of data with regard to quantities traded).

#### Parameter 5 – Importance of the disease for the affected sector (% loss or € lost compared to business amount of the sector)

This cannot be quantified properly but caudata probably represent a minor proportion in the trade.

### **3.1.5.2. Article 7(e)(ii) The societal acceptance of disease prevention and control measures**

Probability of societal acceptance of implementation of mitigation measures is expected to be high, even desired, since mitigation of *Bsal* promotes animal health and welfare and biodiversity conservation. Acceptance will probably depend on the measures imposed and on the explanation/rationale provided for the measures. While biosecurity measures like entry control, disinfection, public education, restricting trade and curative treatments will probably be readily accepted, culling (certainly of wild animals) may provoke a strong and negative response.

### **3.1.5.3. Article 7(e)(iii) The welfare of affected subpopulations of kept and wild animals**

#### Parameter 1 – Welfare impact of control measures on domestic animals

The control measures (biosecurity and treatment) increase welfare of captive caudata since this directly improves animal health. Temperature control should not involve caudata species that cannot tolerate high temperature (25°C check) (see Section 3.1.4.3 parameter on treatment). However, there is a lack of knowledge on the impact of the procedures of treatment on the welfare of salamanders.

#### Parameter 2 – Wildlife depopulation as control measure

Although culling of all caudata in an affected wild community might be theoretically a defensible option in an attempt to eliminate *Bsal* from an infected area, this comes with several, major problems such as: (see also Section 3.1.5.2 above):

- very low probability that depopulation is effective, given the estimated low feasibility of capturing all individuals present at a site;
- the presence of persistent forms of *Bsal* in the environment creating an ongoing risk;
- societal acceptance is most likely very low and adverse reactions from the public, academia and nature conservation organisations can be expected;
- legal issues may prevent depopulation: many European amphibians are strictly protected at national and European level.

### 3.1.5.4. Article 7(e)(iv) The environment and biodiversity

#### Environment

Parameter 1 – Use and potential residuals of biocides or medical drugs in environmental compartments (soil, water, feed, manure)

Manufacture and use of disinfectants in the EU must comply with Regulation (EU) No 528/2012<sup>2</sup>. Specific derogations for the use of disinfectants in the field are necessary (e.g. the use of Virkon, currently routinely used). Disposal of disinfectants in the environment (e.g. chlorine) should be strongly discouraged.

#### Biodiversity

Parameter 2 – Mortality in wild species

The relevance of *Bsal* in the EU is its potential to cause significant mortality in many wild species of caudata (see above). While mortality in captive species can be counteracted, mortality in wild caudata can currently not be mitigated. *Bsal* can drive amphibian species to local extinction. This may lead to loss of biodiversity, at least at local level (Martel et al., 2014; Stegen et al., 2017).

## 3.2. Assessment according to Article 5 criteria

This section presents the results of the expert judgement on the criteria of Article 5 of the AHL about *Bsal* (Table 4). The expert judgement was based on Individual and Collective Behavioural Aggregation (ICBA) approach described in detail in the opinion on the methodology (EFSA AHAW Panel, 2017). Experts have been provided with information of the disease fact-sheet mapped into Article 5 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 5, and the reasoning supporting their judgement.

The minimum number of judges in the judgement was 12. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

**Table 4:** Outcome of the expert judgement on the Article 5 criteria for *Batrachochytrium salamandrivorans*

<b>Criteria to be met by the disease:</b>		<b>Final outcome</b>
According to AHL, a disease shall be included in the list referred to in point (b) of paragraph 1 of Article 5 if it has been assessed in accordance with Article 7 and meets all of the following criteria		
A(i)	The disease is transmissible	Y
A(ii)	Animal species are either susceptible to the disease or vectors and reservoirs thereof exist in the Union	Y
A(iii)	The disease causes negative effects on animal health or poses a risk to public health due to its zoonotic character	Y
A(iv)	Diagnostic tools are available for the disease	Y
A(v)	Risk-mitigating measures and, where relevant, surveillance of the disease are effective and proportionate to the risks posed by the disease in the Union	Y
<b>At least one criterion to be met by the disease:</b>		
In addition to the criteria set out above at points A(i)–A(v), the disease needs to fulfil at least one of the following criteria		
B(i)	The disease causes or could cause significant negative effects in the Union on animal health, or poses or could pose a significant risk to public health due to its zoonotic character	Y
B(ii)	The disease agent has developed resistance to treatments and poses a significant danger to public and/or animal health in the Union	N
B(iii)	The disease causes or could cause a significant negative economic impact affecting agriculture or aquaculture production in the Union	N
B(iv)	The disease has the potential to generate a crisis or the disease agent could be used for the purpose of bioterrorism	N
B(v)	The disease has or could have a significant negative impact on the environment, including biodiversity, of the Union	Y

Colour code: green = consensus (Yes/No).

<sup>2</sup> Regulation (EU) No 528/2012 of the European Parliament and of the Council of 22 May 2012 concerning the making available on the market and use of biocidal products. OJ L 167, 27.6.2012, p. 1–123.

### 3.2.1. Outcome of the assessment of *Batrachochytrium salamandrivorans* according to criteria of Article 5(3) of the AHL on its eligibility to be listed

As from the legal text of the AHL, a disease is considered eligible to be listed as laid down in Article 5 if it fulfils all criteria of the first set from A(i) to A(v) and at least one of the second set of criteria from B(i) to B(v). According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. According to the results shown in Table 4, *Bsal* complies with all criteria of the first set and with two criteria of the second set, therefore it is considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL.

### 3.3. Assessment according to Article 9 criteria

This section presents the results of the expert judgement on the criteria of Annex IV referring to categories as in Article 9 of the AHL about *Bsal* (Tables 5, 6, 7, 8 and 9). The expert judgement was based on ICBA approach described in detail in the opinion on the methodology. Experts have been provided with information of the disease fact-sheet mapped into Article 9 criteria (see supporting information, Annex A), based on that the experts indicate their Y/N or 'na' judgement on each criterion of Article 9, and the reasoning supporting their judgement.

The minimum number of judges in the judgement was 10. The expert judgement was conducted as described in the methodological opinion (EFSA AHAW Panel, 2017). For details on the interpretation of the questions see Appendix B of the methodological opinion (EFSA AHAW Panel, 2017).

**Table 5:** Outcome of the expert judgement related to the criteria of Section 1 of Annex IV (category A of Article 9) for *Batrachochytrium salamandrivorans* (CI: current impact; PI: potential impact)

Criteria to be met by the disease:		Final outcome
The disease needs to fulfil all of the following criteria		
1	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present only in a very limited part of the territory of the Union	NC
2.1	The disease is highly transmissible	NC
2.2	There are possibilities of airborne or waterborne or vector-borne spread	Y
2.3	The disease affects multiple species of kept and wild animals OR single species of kept animals of economic importance	Y
2.4	The disease may result in high morbidity and significant mortality rates	Y
<b>At least one criterion to be met by the disease:</b>		
In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic or pandemic potential OR possible significant threats to food safety	N
4(CI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	N
4(PI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	N
5(a)(CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(a)(PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)(CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC
5(b)(PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	Y
5(c)(CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	Y
5(c)(PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	Y

5(d)(CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	Y
5(d)(PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	Y

Colour code: green = consensus (Yes/No), yellow = non-consensus (NC).

**Table 6:** Outcome of the expert judgement related to the criteria of Section 2 of Annex IV (category B of Article 9) for *Batrachochytrium salamandrivorans* (CI: current impact; PI: potential impact)

<b>Criteria to be met by the disease:</b>		<b>Final outcome</b>
The disease needs to fulfil all of the following criteria		
1	The disease is present in the whole OR part of the Union territory with an endemic character AND (at the same time) several Member States or zones of the Union are free of the disease	NC
2.1	The disease is moderately to highly transmissible	NC
2.2	There are possibilities of airborne or waterborne or vector-borne spread	Y
2.3	The disease affects single or multiple species	Y
2.4	The disease may result in high morbidity with in general low mortality	N
<b>At least one criterion to be met by the disease:</b>		
In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, including epidemic potential OR possible significant threats to food safety	N
4(CI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	N
4(PI)	The disease has a significant impact on the economy of the Union, causing substantial costs, mainly related to its direct impact on the health and productivity of animals	N
5(a)(CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(a)(PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)(CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC
5(b)(PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	Y
5(c)(CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	Y
5(c)(PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	Y
5(d)(CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	Y
5(d)(PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	Y

Colour code: green = consensus (Yes/No), yellow = non-consensus (NC).

**Table 7:** Outcome of the expert judgement related to the criteria of Section 3 of Annex IV (category C of Article 9) for *Batrachochytrium salamandrivorans* (CI: current impact; PI: potential impact)

<b>Criteria to be met by the disease:</b> The disease needs to fulfil all of the following criteria		<b>Final outcome</b>
1	The disease is present in the whole OR part of the Union territory with an endemic character OR in aquatic animals several Member States or zones of the Union are free of the disease	Y
2.1	The disease is moderately to highly transmissible	NC
2.2	The disease is transmitted mainly by direct or indirect transmission	Y
2.3	The disease affects single or multiple species	Y
2.4	The disease usually does not result in high morbidity and has negligible or no mortality AND often the most observed effect of the disease is production loss OR in aquatic animals the disease may result in high morbidity and usually low mortality AND often the most observed effect of the disease is production loss	N
<b>At least one criterion to be met by the disease:</b> In addition to the criteria set out above at points 1–2.4, the disease needs to fulfil at least one of the following criteria		
3	The disease has a zoonotic potential with significant consequences on public health, or possible significant threats to food safety	N
4(CI)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	N
4(PI)	The disease has a significant impact on the economy of parts of the Union, mainly related to its direct impact on certain types of animal production systems	N
5(a)(CI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(a)(PI)	The disease has a significant impact on society, with in particular an impact on labour markets	N
5(b)(CI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC
5(b)(PI)	The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	Y
5(c)(CI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	Y
5(c)(PI)	The disease has a significant impact on the environment, due to the direct impact of the disease OR due to the measures taken to control it	Y
5(d)(CI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	Y
5(d)(PI)	The disease has a significant impact on a long-term effect on biodiversity or the protection of endangered species or breeds, including the possible disappearance or long-term damage to those species or breeds	Y

Colour code: green = consensus (Yes/No), yellow = non-consensus (NC).

**Table 8:** Outcome of the expert judgement related to the criteria of Section 4 of Annex IV (category D of Article 9) for *Batrachochytrium salamandrivorans*

<b>Criteria to be met by the disease:</b> The disease needs to fulfil all of the following criteria		<b>Final outcome</b>
D	The risk posed by the disease in question can be effectively and proportionately mitigated by measures concerning movements of animals and products in order to prevent or limit its occurrence and spread	Y
The disease fulfils criteria of Sections 1, 2, 3 or 5 of Annex IV of AHL		Y

Colour code: green = consensus (Yes/No).



**Table 9:** Outcome of the expert judgement related to the criteria of Section 5 of Annex IV (category E of Article 9) for *Batrachochytrium salamandrivorans*

Diseases in category E <b>need to fulfil criteria of Sections 1, 2 or 3 of Annex IV of AHL</b> and/or the following:		Final outcome
E	Surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment (If a disease fulfils the criteria as in Article 5, thus being eligible to be listed, consequently category E would apply.)	Y

Colour code: green = consensus (Yes/No).

### 3.3.1. Non-consensus-questions

This section displays the assessment related to each criterion of Annex IV referring to the categories of Article 9 of the AHL where no consensus was achieved in form of Tables 10–12. The proportion of Y, N or 'na' answers are reported, followed by the list of different supporting views for each answer.

**Table 10:** Outcome of the expert judgement related to criterion 1 of Article 9

Question		Final outcome	Response		
			Y (%)	N (%)	na (%)
1 (cat. A)	The disease is not present in the territory of the Union OR present only in exceptional cases (irregular introductions) OR present only in a very limited part of the territory of the Union	NC	50	50	0
1 (cat. B)	The disease is present in the whole OR part of the Union territory with an endemic character AND (at the same time) several Member States or zones of the Union are free of the disease	NC	50	50	0

NC: non-consensus; number of judges: 12.

#### Reasoning supporting the judgement

Supporting Yes for 1 (cat. A):

- The distribution of *Bsal* is currently very limited, and its presence is still not endemic, although very likely to become, since it will be difficult to avoid spread further given multiple outbreaks in wild species already in diverse locations.

Supporting Yes for 1 (cat. B):

- It is present in wild populations in Belgium, Germany, the Netherlands (Spitzen-van der Sluijs et al., 2016) and in captive salamanders in Germany, the Netherlands, Spain and the United Kingdom (Fitzpatrick et al., 2016; Sabino-Pinto et al., 2015).
- It appears that the disease can maintain itself in the population without introduction from outside.

**Table 11:** Outcome of the expert judgement related to criterion 2.1 of Article 9

Question		Final outcome	Response		
			Y (%)	N (%)	na (%)
2.1 (cat. A)	The disease is highly transmissible	NC	33	67	0
2.1 (cat. B, C)	The disease is moderately to highly transmissible	NC	67	33	0

NC: non-consensus; number of judges: 12.

### Reasoning supporting the judgement

Supporting Yes for 2.1 (cat. A):

- It has caused rapid population crashes in both captive and wild populations.
- Salamanders carrying high-infection loads can spread *Bsal* infection to naïve salamanders within 2 h of cohousing.

Supporting Yes for 2.1 (cat. B, C):

- It is moderately to highly transmissible according to the host species and the infectious dose.
- Within certain wetlands, if animals have close contact, the transmissibility can be very high. A different situation occurs between separated wetlands, where the disease has lower transmissibility, due to the segregation of amphibian population.

**Table 12:** Outcome of the expert judgement related to criterion 5(b)(CI) of Article 9

Question	Final outcome	Response		
		Y (%)	N (%)	na (%)
5b The disease has a significant impact on animal welfare, by causing suffering of large numbers of animals	NC	83	17	0

NC: non-consensus; number of judges: 12.

### Reasoning supporting the judgement

Supporting Yes:

- *Bsal* infection leads to a lethal, ulcerative skin disease with high mortality and a clear welfare impact.
- Morbidity is high in some species of European caudata, the salamander population in at least three MSs is affected, so it could be considered a large number of animals.

Supporting No:

- The number of affected animals is not large considering that *Bsal* has been detected in few locations in Belgium (5), Germany (3) and Netherlands (7).

### 3.3.2. Outcome of the assessment of criteria in Annex IV for *Batrachochytrium salamandrivorans* for the purpose of categorisation as in Article 9 of the AHL

As from the legal text of the AHL, a disease is considered fitting in a certain category (A, B, C, D or E corresponding to point (a) to point (e) of Article 9(1) of the AHL) if it is eligible to be listed for Union intervention as laid down in Article 5(3) and fulfils all criteria of the first set from 1 to 2.4 and at least one of the second set of criteria from 3 to 5(d) as shown in Tables 5–9. According to the assessment methodology (EFSA AHAW Panel, 2017), a criterion is considered fulfilled when the outcome is 'Yes'. With respect to different type of impact where the assessment is divided into current and potential impact, a criterion will be considered fulfilled if at least one of the two outcomes is 'Y' and, in case of no 'Y', the assessment is inconclusive if at least one outcome is 'NC'.

A description of the outcome of the assessment of criteria in Annex IV for *Bsal* for the purpose of categorisation as in Article 9 of the AHL is presented in Table 13.

**Table 13:** Outcome of the assessment of criteria in Annex IV for *Batrachochytrium salamandrivorans* for the purpose of categorisation as in Article 9 of the AHL

Category	Article 9 criteria										
	1° set of criteria					2° set of criteria					
	1	2.1	2.2	2.3	2.4	3	4	5a	5b	5c	5d
	Geographical distribution	Transmissibility	Routes of transmission	Multiple species	Morbidity and mortality	Zoonotic potential	Impact on economy	Impact on society	Impact on animal welfare	Impact on environment	Impact on biodiversity
A	NC	NC	Y	Y	Y	N	N	N	Y	Y	Y
B	NC	NC	Y	Y	N	N	N	N	Y	Y	Y
C	Y	NC	Y	Y	N	N	N	N	Y	Y	Y
D	Y										
E	Y										

According to the assessment here performed, *Bsal* complies with the following criteria of the Sections 1–5 of Annex IV of the AHL for the application of the disease prevention and control rules referred to in points (a) to (e) of Article 9(1):

- 1) To be assigned to category A, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment *Bsal* complies with criteria 2.2, 2.3 and 2.4 and the assessment is inconclusive on compliance with criteria 1 and 2.1. To be eligible for category A, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and *Bsal* complies with criteria 5b, 5c and 5d, but not with criteria 3, 4 and 5a.
- 2) To be assigned to category B, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment *Bsal* complies with criteria 2.2 and 2.3, but not with criterion 2.4 and the assessment is inconclusive on compliance with criteria 1 and 2.1. To be eligible for category B, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and *Bsal* complies with criteria 5b, 5c and 5d, but not with criteria 3, 4 and 5a.
- 3) To be assigned to category C, a disease needs to comply with all criteria of the first set (1, 2.1–2.4) and according to the assessment *Bsal* complies with criteria 1, 2.2 and 2.3, but not with criterion 2.4 and the assessment is inconclusive on compliance with criterion 2.1. To be eligible for category C, a disease needs to comply additionally with one of the criteria of the second set (3, 4, 5a–d) and *Bsal* complies with criteria 5b, 5c and 5d, but not with criteria 3, 4 and 5a.
- 4) To be assigned to category D, a disease needs to comply with criteria of Sections 1, 2, 3 or 5 of Annex IV of the AHL and with the specific criterion D of Section 4, with which *Bsal* complies.
- 5) To be assigned to category E, a disease needs to comply with criteria of Sections 1, 2 or 3 of Annex IV of the AHL and/or the surveillance of the disease is necessary for reasons relating to animal health, animal welfare, human health, the economy, society or the environment. The latter is applicable if a disease fulfils the criteria as in Article 5, with which *Bsal* complies.

### 3.4. Assessment of Article 8

This section presents the results of the assessment on the criteria of Article 8(3) of the AHL about *Bsal*. The Article 8(3) criteria are about animal species to be listed, as it reads below:

'3. Animal species or groups of animal species shall be added to this list if they are affected or if they pose a risk for the spread of a specific listed disease because:

- a) they are susceptible for a specific listed disease or scientific evidence indicates that such susceptibility is likely; or
- b) they are vector species or reservoirs for that disease, or scientific evidence indicates that such role is likely<sup>3</sup>.

For this reason, the assessment on Article 8 criteria is based on the evidence as extrapolated from the relevant criteria of Article 7, i.e. the ones related to susceptible and reservoir species or routes of transmission, which cover also possible role of biological or mechanical vectors.<sup>3</sup> According to the mapping, as presented in Table 5, Section 3.2 of the scientific opinion on the ad hoc methodology (EFSA AHAW Panel, 2017), the main animal species to be listed for *Bsal* according to the criteria of Article 8(3) of the AHL are as displayed in Table 14.

**Table 14:** Main animal species to be listed for *Batrachochytrium salamandrivorans* according to criteria of Article 8 (source: data reported in Section 3.1.1.1)

	Class	Order	Family	Common names (Genus/Species)
<b>Susceptible</b>	Amphibia	Caudata	Salamandridae	Fire salamander ( <i>Salamandra salamandra</i> ), Alpine newt ( <i>Ichthyosaura alpestris</i> ), smooth newt ( <i>Lissotriton vulgaris</i> ), North African fire salamander ( <i>Salamandra algira</i> ), Corsican fire salamander ( <i>Salamandra corsica</i> ), Near Eastern fire salamander ( <i>Salamandra infraimmaculata</i> ), Macedonian crested newt ( <i>Triturus macedonicus</i> ), marbled newt ( <i>Triturus marmoratus</i> ), Eastern newt ( <i>Notophthalmus viridescens</i> ), rough-skinned newt ( <i>Taricha granulosa</i> ), Italian newt ( <i>Lissotriton italicus</i> ), Northern crested newt ( <i>Triturus cristatus</i> ), spectacled salamander ( <i>Salamandrina terdigitata</i> ), yellow-spotted newt ( <i>Neurergus crocatus</i> ), Sardinian mountain newt ( <i>Euproctus platycephalus</i> ), <i>Chioglossa</i> spp., <i>Lyciasalamandra</i> spp., <i>Mertensiella</i> spp., <i>Ommatotriton</i> spp., <i>Paramesotriton</i> spp., <i>Hypselotriton</i> spp.
			Plethodontidae	Northern slimy salamander ( <i>Plethodon glutinosus</i> )
		Caudata	Plethodontidae	French cave salamander ( <i>Speleomantes strinati</i> )
			Salamandridae	Iberian ribbed newt ( <i>Pleurodeles waltl</i> ), <i>Tylosotriton</i> spp., <i>Cynops</i> spp.
<b>Reservoir</b>	Amphibia	Caudata	Salamandridae	Japanese fire belly newt ( <i>Cynops pyrrhogaster</i> ), sword-tailed newt ( <i>Cynops ensicauda</i> ), Vietnamese crocodile newt ( <i>Tylosotriton vietnamensis</i> ), black knobby newt ( <i>Tylosotriton asperrimus</i> ), Chiang Mai crocodile newt ( <i>Tylosotriton uyenoi</i> ), Tam Dao salamander ( <i>Paramesotriton deloustali</i> ), Alpine newt ( <i>Ichthyosaura alpestris</i> )
			Hynobiidae	Siberian salamander ( <i>Salamandrella keyserlingii</i> ), clouded salamander ( <i>Hynobius nebulosus</i> ), Japanese clawed salamander ( <i>Onychodactylus japonicus</i> )
		Caudata	Salamandridae	Ziegler's crocodile newt ( <i>Tylosotriton zieglerei</i> )
		Anura	Alytidae	Midwife toads ( <i>Alytes obstetricans</i> )
<b>Vectors</b>	None			

## 4. Conclusions

**TOR 1:** for each of those diseases an assessment, following the criteria laid down in Article 7 of the AHL, on its eligibility of being listed for Union intervention as laid down in Article 5(3) of the AHL;

<sup>3</sup> A vector is a living organism that transmits an infectious agent from an infected animal to a human or another animal. Vectors are frequently arthropods. Biological vectors may carry pathogens that can multiply within their bodies and be delivered to new hosts, usually by biting. In mechanical vectors, the pathogens do not multiply within the vector, which usually remains infected for shorter time than in biological vectors.

- According to the assessment here performed, *Bsal* complies with all criteria of the first set and with two criteria of the second set and therefore can be considered eligible to be listed for Union intervention as laid down in Article 5(3) of the AHL.

**TOR 2a:** for each of the diseases which was found eligible to be listed for Union intervention, an assessment of its compliance with each of the criteria in Annex IV to the AHL for the purpose of categorisation of diseases in accordance with Article 9 of the AHL;

- According to the assessment here performed, *Bsal* meets the criteria as in Sections 4 and 5 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in points (d) and (e) of Article 9(1) of the AHL. According to the assessment here performed, it is inconclusive whether *Bsal* complies with the criteria as in Section 1 of Annex IV of the AHL, for the application of the disease prevention and control rules referred to in point (a) of Article 9(1) of the AHL. Compliance of *Bsal* with the criteria as in Section 1 is dependent on a decision on criteria 1 and 2.1.

**TOR 2b:** for each of the diseases which was found eligible to be listed for Union intervention, a list of animal species that should be considered candidates for listing in accordance with Article 8 of the AHL.

- According to the assessment here performed, the animal species that can be considered to be listed for *Bsal* according to Article 8(3) of the AHL are species of the families Salamandridae and Plethodontidae as susceptible and reservoirs, as reported in Table 14 in Section 3.4 of the present document.

## References

- AVMA (American Veterinary Medical Association), 2013. AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. Schaumburg, Illinois, 102 pp. Available online: <https://www.avma.org/KB/Policies/Documents/euthanasia.pdf>
- Beukema W, Martel A, Nguyen TT, Goka K, Schmeller DS, Yuan Z, Laking AE, Nguyen TQ, Lin CF, Sheldon J, Loyau A and Pasmans F, 2017. Colonisation and spread of *Batrachochytrium salamandrivorans* in the Western Palearctic is governed by changes in pathogen niche structure and environmental context. Unpublished document.
- Blooi M, Pasmans F, Longcore JE, Spitzen-van der Sluijs A, Vercammen F and Martel A, 2013. Duplex real-time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in Amphibian samples. *Journal of Clinical Microbiology*, 51, 4173–4177.
- Blooi M, Martel A, Haesebrouck F, Vercammen F, Bonte D and Pasmans F, 2015a. Treatment of caudata based on temperature dependent infection dynamics of *Batrachochytrium salamandrivorans*. *Scientific Reports*, 5, 8037.
- Blooi M, Pasmans F, Rouffaer L, Haesebrouck F, Vercammen F and Martel A, 2015b. Successful treatment of *Batrachochytrium salamandrivorans* infections in salamanders requires synergy between voriconazole, polymyxin E and temperature. *Scientific Reports*, 5, 11788.
- Brown LD, Cai TT and DasGupta A, 2001. Interval estimation for a binomial proportion. *Statistical Science*, 16, 101–117.
- Canessa S, Bozzuto C, Cruickshank S, Fisher M, Koella J, Lötters S, Martel A, Pasmans F, Spitzen-van der Sluijs A, Steinfartz S and Schmidt B, 2017. Decision making for mitigating emerging wildlife diseases: from theory to practice. Submitted.
- CFSPH (Center for Food Security and Public Health), online, 2017. List of zoonotic diseases. Available online: <http://www.cfsph.iastate.edu/Products/bioterrorism-and-high-consequence-pathogen-wallchart.php> [Accessed: 8 September 2017]
- DGHT (Deutsche Gesellschaft für Herpetologie und Terrarienkunde), 2016. Handlungsempfehlungen zum Umgang mit dem Salamanderpilz *Batrachochytrium salamandrivorans* (Bsal). Positionspapier der Deutschen Gesellschaft für Herpetologie und Terrarienkunde (DGHT e.V.).
- Dillon MJ, Bowkett AE, Bungard MJ, Beckman KM, O'Brien MF, Bates K, Fisher MC, Stevens JR and Thornton CR, 2017. Tracking the amphibian pathogens *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* using a highly specific monoclonal antibody and lateral-flow technology. *Microbial Biotechnology*, 10, 381–394.
- EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), More S, Bøtner A, Butterworth A, Calistri P, Depner K, Edwards S, Garin-Bastuji B, Good M, Gortázar Schmidt C, Michel V, Miranda MA, Nielsen SS, Raj M, Sihvonen L, Spooler H, Stegeman JA, Thulke H-H, Velarde A, Willeberg P, Winckler C, Baldinelli F, Broglia A, Candiani D, Gervelmeyer A, Zancanaro G, Kohnle L, Morgado J and Bicot D, 2017. Scientific opinion on an ad hoc method for the assessment on listing and categorisation of animal diseases within the framework of the Animal Health Law. *EFSA Journal* 2017;15(5):4783, 42 pp. <https://doi.org/10.2903/j.efsa.2017.4783>



- EFSA (European Food Safety Authority), Balaz V, Gortazar Schmidt C, Murray K, Carnesecchi E, Garcia A, Gervelmeyer A, Martino L, Munoz Guajardo I, Verdonck F, Zancanaro G and Fabris C, 2017. Scientific report: scientific and technical assistance concerning the survival, establishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. EFSA Journal 2017;15(2):4739, 73 pp. <https://doi.org/10.2903/j.efsa.2017.4739>
- Fitzpatrick L, Pasmans F, Martel A and Cunningham AA, 2016. Epidemiological tracing of *Batrachochytrium salamandrivorans* infection in European private amphibian collections. Proceedings of the European Wildlife Disease Association Conference 2016, Berlin, Germany.
- Gilbert M, Spitzen-van der Sluijs A and Zollinger R, 2016. *Surveillance naar Batrachochytrium salamandrivorans in de handel*. Stichting RAVON, Nijmegen.
- Gvozdk L, Puky M, Sugerova M, 2007. Acclimation is beneficial at extreme test temperatures in the Danube crested newt, *Triturus dobrogicus* (Caudata, Salamandridae). Biological Journal of the Linnean Society, 90, 627–636.
- Herrel A and van der Meijden A, 2014. An analysis of the live reptile and amphibian trade in the USA compared to the global trade in endangered species. Herpetological Journal, 24, 103–110.
- Johnson ML, Berger L, Philips L and Speare R, 2003. Fungicidal effects of chemical disinfectants, UV light, desiccation and heat on the amphibian chytrid *Batrachochytrium dendrobatidis*. Diseases of Aquatic Organisms, 57, 255–260.
- Joint Response, 2017. Addressing the spread of the BSAL pathogen: an assessment of the trade and supply chain process for newts and salamanders entering the EU. A joint response submitted to the European Commission in April 2017 by Ornamental Fish International (OFI, The Netherlands), Ornamental Aquatic Trade Association (OATA, UK), the Reptile and Exotic Pet Trade Association (REPTA, UK) and the Pet Industry Federation (PIF, UK).
- Laking AE, Ngo HN, Pasmans F, Martel A and Nguyen TT, 2017. *Batrachochytrium salamandrivorans* is the predominant chytrid fungus in Vietnamese salamanders. Scientific Reports, 7, 44443.
- Martel A and Pasmans F, 2017. Personal communication to EFSA. October 2017.
- Martel A, Spitzen-van der Sluijs A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F and Pasmans F, 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proceedings of the National Academy of Sciences, 110, 15325–15329.
- Martel A, Blooi M, Adriaensen C, Van Rooij P, Beukema W, Fisher MC, Farrer RA, Schmidt BR, Tobler U, Goka K, Lips KR, Mulet C, Zamudio KR, Bosch J, Lötters S, Wombwell E, Garner TWJ, Cunningham AA, Spitzen-van der Sluijs A, Salvidio S, Ducatelle R, Nishikawa K, Nguyen TT, Kolby JE, Van Bocxlaer I, Bossuyt F and Pasmans F, 2014. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders. Science, 346, 630–631.
- Sabino-Pinto J, Bletz M, Hendrix R, Perl RGB, Martel A, Pasmans F, Lötters S, Mutschmann F, Schmeller DS, Schmidt BR, Veith M, Wagner N, Vences M and Steinfartz S, 2015. First detection of the emerging fungal pathogen *Batrachochytrium salamandrivorans* in Germany. Amphibia-Reptilia, 36, 411–416.
- Shine R, Amiel J, Munn AJ, Stewart M, Vyssotski AL and Lesku JA, 2015. Is “cooling then freezing” a humane way to kill amphibians and reptiles? Biology Open, 4, 760–763.
- Spitzen-van der Sluijs AM, Bogaerts S, Woeltjes T, Pasmans F and Martel A, 2015. Rapport Pathway Analyse Chytrid 2.0. 52 pp. Available online: <http://www.ravon.nl/Portals/0/PDFx/Pathway%20Analyse%20Chytrid%202.0%20.pdf>
- Spitzen-van der Sluijs A, Martel A, Asselberghs J, Bales EK, Beukema W, Bletz MC, Dalbeck L, Goverse E, Kerres A, Kinet T, Kirst K, Laudelout A, Marin da Fonte LF, Nöllert A, Ohlhoff D, Sabino-Pinto J, Schmidt BR, Speybroeck J, Spikmans F, Steinfartz S, Veith M, Vences M, Wagner N, Pasmans F and Lötters S, 2016. Expanding distribution of lethal amphibian fungus *Batrachochytrium salamandrivorans* in Europe. Emerging Infectious Diseases, 22, 1286–1288.
- Stegen G, Pasmans F, Schmidt BR, Rouffaer LO, Van Praet S, Schaub M, Canessa S, Laudelout A, Kinet T, Adriaensen C, Haesebrouck F, Bert W, Bossuyt F and Martel A, 2017. Drivers of salamander extirpation mediated by *Batrachochytrium salamandrivorans*. Nature, 544, 353–356.
- Thomas V, Blooi M, Van Rooij P, Van Praet S, Verbrugghe E, Grasselli E, Lukac M, Smith S, Pasmans F and Martel A, submitted. Recommendations on diagnostic tools for *Batrachochytrium salamandrivorans*. Submitted to Transboundary and Emerging Diseases on 13 September 2017.
- US Fish and Wildlife Service, 2016. Listing salamanders as injurious due to risk of salamander chytrid fungus. Available online: <http://www.fws.gov/injuriouswildlife/salamanders.html>
- Van Rooij P, Martel A, Haesebrouck F and Pasmans F, 2015. Amphibian chytridiomycosis: a review with focus on fungus-host interactions. Veterinary Research, 46, 137.
- Van Rooij P, Pasmans F, Coen Y and Martel A, 2017. Efficacy of chemical disinfectants for the containment of the salamander chytrid fungus *Batrachochytrium salamandrivorans*. Unpublished document.
- Warburg MR, 1971. The water economy of Israel amphibians: the urodeles *Triturus vittatus* (jenyns) and *Salamandra salamandra*. Comparative Biochemistry and Physiology, 1055–1063.
- White C, Forzan M, Pessier A, Allender M, Ballard J, Catenazzi A, Fenton H, Martel A, Pasmans F, Miller DL, Ossiboff RJ, Richgels K and Kerby J, 2016. Amphibian: a case definition and diagnostic criteria for *Batrachochytrium salamandrivorans* Chytridiomycosis. Herpetological Review, 47, 207–209.
- Yap TA, Koo MS, Ambrose RF, Wake DB and Vredenburg VT, 2015. Averting a North American biodiversity crisis. Science, 349, 481–482.

## Abbreviations

AHL	AHLAnimal Health Law
<i>Bsal</i>	<i>Batrachochytrium salamandrivorans</i>
Bd	<i>Batrachochytrium dendrobatidis</i>
CFSPH	Center for Food Security and Public Health
CITES	Convention on International Trade in Endangered Species
DGHT	Deutsche Gesellschaft für Herpetologie und Terrarienkunde
DSe	diagnostic sensitivity
DSp	diagnostic specificity
GE	genomic equivalents
ICBA	Individual and Collective Behavioural Aggregation
IUCN	International Union for Conservation of Nature
MS	Member States
OIE	World Organisation for Animal Health
qPCR	quantitative polymerase chain reaction
ToR	Terms of Reference